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EVIDENCE REVIEW

Evidence-based approach to the treatment of esophagogastric junction tumors

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Abstract

The incidence of esophagogastric junction (EGJ) adenocarcinoma is increasing in developed nations due to the rising prevalence of obesity and gastroesophageal reflux disease. Due to the peculiar location in a histological transition zone between the esophagus and the stomach, the management of EGJ tumors is controversial. Two main surgical approaches exist: total gastrectomy with distal esophagectomy or esophagectomy by either transhiatal or transthoracic approach. These operations differ significantly in the extent of lymphadenectomy. In addition, patients with locally advanced disease can receive either preoperative chemoradiation or perioperative chemotherapy. This evidence-based review analyzes current evidence regarding the management of EGJ tumors in order to help defining the best surgical and systemic treatment of these patients.

Key Words: Esophagogastric junction tumors; Esophagectomy; Gastrectomy; Esophageal adenocarcinoma; Chemotherapy; Chemoradiation

Core Tip: Management of patients with esophagogastric junction tumors is challenging. Several surgical approaches and systemic therapies are currently available to treat these patients. This evidence-based review will help determining the optimal treatment for this complex disease.

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INTRODUCTION

Adenocarcinoma of the esophagogastric junction (EGJ) remains a major global health problem associated with poor prognosis[1]. The majority of patients are diagnosed at an advanced stage, and only half of the patients undergo curative treatment[2]. EGJ tumors arise in the histological transition area between the esophagus and the stomach. This zone is vulnerable to gastric acid reflux and consequently has an increased risk of malignant transformation[3]. The incidence of EGJ tumors vary among countries, but it has been increasing in the past years due to the rising prevalence of obesity and gastroesophageal reflux disease in developed nations[4,5].

Siewert described three types of EGJ tumors based on the relationship of the epicenter of the tumor and the endoscopic location of the Z line (*i.e.*, squamocolumnar junction): type I (distal esophageal tumors) when the epicenter is 1-5 cm above Z line, type II (true EGJ tumors) from 1 over to 2 cm below the Z line, and type III (subcardial tumors) when the epicenter is 2-5 cm distal to the Z line[6]. The Nishi classification is also based on where the center of the tumor is located; there are 5 types depending on the relative extent of the esophageal or gastric involvement (E, EG, E=G, GE, and G) and true EGJ tumors are represented by EG, E=G or GE[7].

Both Siewert and Nishi classifications describe the location of the center of the lesion, but do not consider the proximal or distal extent of the tumor, which is more relevant to guide the extent of surgical resection. In addition, the lymphatic drainage of EGJ cancers is variable. Specifically, type II tumors can metastasize to either paraoesophageal nodes in the lower mediastinum or upper abdominal lymph nodes[8].

Two main surgical approaches for EGJ tumors exist: total gastrectomy with distal esophagectomy and esophagectomy by either transhiatal or transthoracic approach[9,10].

Both operations allow for adequate dissection of para-celiac and para-aortic lymph nodes. However, better mediastinal lymph node dissection and larger proximal resection margins can be achieved with an esophagectomy[11,12].

Some oncological and surgical principles that are well-established for esophageal and gastric tumors cannot be simply applied to junctional cancers due to their specific location and pathological features. The aim of this study was to review the available evidence in an attempt to determine the optimal treatment for patients with EGJ tumors.

SURGICAL TREATMENT OF EGJ TUMORS

Esophagectomy remains the cornerstone of curative treatment of esophageal cancer. The goals of the operation are to achieve a resection with clear margins, with an adequate lymphadenectomy, and with acceptable morbidity and mortality rates in order to offer better long-term survival.

Surgical approach

R0 resection remains one of the most important prognostic factors for survival irrespectively of the tumor type or surgical approach. Consequently, technical considerations regarding proximal margin should influence surgical strategy. Most experts base their surgical approach on Siewert classification, recommending an esophagogastrectomy for type I tumors and a total gastrectomy for type III tumors. However, the main debate arises for type II lesions: esophagectomy or gastrectomy?

Different approaches are proposed for true cardia tumors. Some authors support esophagectomy because it allows an extensive mediastinal lymph node resection along with a longer proximal resection margin that may decrease the likelihood of microscopically positive margins. On the other hand, a total gastrectomy with distal esophagectomy may be preferred because it avoids entering the chest, and an adequate abdominal lymph node dissection can be achieved (potentially the most important nodes in these patients).

Esophagectomy and gastrectomy are significantly different in terms of invasiveness, type of reconstruction, and, more importantly, extent of gastric and esophageal resection. After analyzing 1002 consecutive patients undergoing surgery for EGJ cancers, Siewert *et al*[13] concluded that in patients with type II EGJ tumors an esophagectomy offers no advantage over an extended gastrectomy if a complete tumor resection can be achieved. No differences were observed in R0 resection rates or number of lymph nodes removed. In addition, esophagectomy was associated with higher 30-d mortality when compared with total gastrectomy[13].

Barbour *et al*[11] evaluated whether the length of esophageal resection or the operative approach influences the outcomes in patients with EGJ tumors. They analyzed 153 patients undergoing gastrectomy and 352 esophagectomy. No differences were found regarding lymph nodes harvested, R0 resection rates, or mortality between groups. Gastrectomy was indeed associated with shorter proximal margins than those undergoing esophagectomy for each Siewert type. Improved outcomes were seen with an esophageal margin > 3.8 cm. The authors concluded that if an adequate proximal margin is achieved, the operative approach might not modify overall survival[11]. Another study, which included 266 patients with surgically resected type II EGJ tumors, found that gastrectomy was more frequently associated with a positive circumferential resection margin than esophagectomy (29% *vs* 11%; $P = 0.025$) [14]. Considering how critical is to achieve adequate proximal margins in these patients, we strongly believe that a gastrectomy should only be considered if a large proximal margin is feasible.

Another matter of debate are the morbidity and mortality rates associated with the different surgical approaches proposed for EGJ tumors. A previous study compared patients undergoing thoracoabdominal esophagectomy ($n = 56$) with transhiatal extended gastrectomy ($n = 186$); this study did not find significant differences regarding perioperative morbidity, anastomotic leak rates, pulmonary complications, or mortality[15]. Another study analyzed two large databases, including 4996 patients with type II EGJ tumors, which found similar major postoperative morbidity (34% *vs* 33%; $P = 0.84$) and 30-d mortality (1.9% *vs* 3.4%; $P = 0.24$) with the esophageal and gastric approach. In addition, the surgical approach was not an independent predictor of overall survival[16]. These findings were supported by other authors[17-19]. Nevertheless, postoperative morbidity after an esophagectomy remains high[20]. In an effort to decrease morbidity, minimally invasive esophagectomy (MIE) has been widely adopted in the last decades[21]. For instance, the TIME trial was the first randomized trial comparing patients undergoing MIE or open esophagectomy and showed that postoperative pulmonary infections rates significantly decrease after MIE. Also, shorter length of stay and better quality of life were achieved in the MIE group[22].

Lymphadenectomy

Lymph node metastasis is another critical prognostic factor in patients with esophageal adenocarcinoma. Therefore, another major goal of the operation is to perform an adequate lymphadenectomy. As an increased number of metastatic lymph nodes is predictive of poor survival, an extensive lymphadenectomy is recommended by the American Joint Committee on Cancer in order to achieve accurate N staging[23]. However, whether extensive lymphadenectomy can improve overall survival because of better control of locoregional disease or better staging remains unclear. In addition, an extensive lymphadenectomy may potentially increase surgical morbidity.

Many studies have tried to determine how many nodes should be removed in patients with EGJ tumors for achieving optimal oncological outcomes[24-27]. For instance, Samson *et al*[26] found that sampling 15 or more lymph nodes was independently associated with lower overall mortality. Moreover, overall survival was improved when more than 20-25 lymph nodes were sampled even in patients with negative nodes, probably due to an increased staging accuracy[26]. This finding was also supported by other authors[25,28]. Greenstein *et al*[29] found that in patients with T2/T3 tumors, better survival rates were observed when more than 10 lymph nodes retrieved, and for T1 tumors, more than 18 lymph nodes were needed for superior survival rates. A recent study recommends the removal of at least 15 lymph nodes in both primary surgery and after induction therapy[24]. Sihag *et al*[28] analyzed 778 patients with locally advanced esophageal adenocarcinoma and found that overall and disease-free survival improved when harvesting up to 20-25 lymph nodes. A lower number of lymph node resection was independently associated with worse overall and disease-free survival[28].

Overall nodal metastasis rate in EGJ tumors varies among the literature between 40% and 80%[30-32]. The EGJ has two main lymphatic drainage pathways: abdominal and mediastinal. Mediastinal lymph nodes involvement varies between 15%-45% in the literature[31,32]. Siewert *et al*[13] evaluated the pattern of lymphatic spread specifically in type II EGJ cancers and showed that almost 70% of the tumors spread towards paracardial, lesser curvature, and left gastric artery nodes while only 15% towards lymphatic nodes in lower posterior mediastinum. However, as all patients underwent a gastrectomy, upper mediastinal nodes were not evaluated in these patients.

Leers *et al*[30] analyzed patients with distal esophageal and EGJ tumors undergoing an esophagectomy with systematic mediastinal and upper abdominal lymphadenectomy. The authors found that 26% of the distal esophageal tumors and 25% of the EGJ tumors had positive mediastinal nodes. Moreover, in 9% and 8% of the patients, respectively, this location was the only site of nodal involvement, concluding that mediastinal node dissection was essential in the surgical therapy for EGJ tumors[30].

Yamashita *et al*[33] recently showed that nodal metastasis in EGJ tumors more frequently involve abdominal nodes, especially those at the right and left cardia, lesser curvature, and along the left gastric artery.

A recent study showed higher incidence of metastasis or recurrence in the upper and middle mediastinal zones when the esophageal invasion length was more than 25 mm[34]. These findings were supported by a Japanese prospective study that included 363 patients undergoing either gastrectomy by a transhiatal approach or distal esophagectomy by a right transthoracic approach. The authors

concluded that upper and lower mediastinal station dissections should be performed in cases of more than 4 cm or 2 cm of esophageal involvement, respectively[35]. Conversely, routine dissection of lymph nodes at the lesser curvature and along the left gastric artery for any EGJ tumor was recommended.

A randomized trial was conducted to compare extended transthoracic resection with limited transhiatal resection for Siewert type I and II. Although a higher number of lymph nodes were harvested through the transthoracic approach (31 *vs* 16), the 5-year survival was similar between groups (34% *vs* 36%). A subgroup analysis was also performed for type I tumors, and a survival benefit of 14% was achieved with the transthoracic approach (51% *vs* 37%). The authors concluded that in type I tumors the transthoracic approach might have survival advantages, especially in those with 1 to 8 positive nodes in the resection specimen[36]. Parry *et al*[14] also showed that a better mediastinal lymph node resection was achieved with an esophagectomy, and these results were supported by other authors [11,37,38].

Advanced techniques to optimize intraoperative lymphadenectomy have been developed in the last decades. For instance, the indocyanine green (ICG) fluorescence imaging for the evaluation of lymph node involvement has increasingly been used, and it might help guiding lymphadenectomy. The goal of this technology is to sample specific tumor-associated lymph nodes and increase pathological evaluation of more likely affected nodes. A targeted lymphadenectomy might provide more accurate and relevant prognostic information and may potentially decrease operative time and reduce postoperative complications. For instance, a previous study evaluated the lymphatic drainage pattern in patients with distal esophageal or EGJ cancer and found that in 89% of the cases, the first nodal station was along the left gastric artery. Interestingly, all patients with nodal involvement had positive nodes in the first nodal station identified with ICG[39]. Therefore, histopathological examination of the first nodal station might avoid unnecessary extensive lymphadenectomy. Further studies are needed to determine how fluorescence imaging can guide lymphadenectomy during an esophagectomy.

Expert commentary

Current evidence shows that surgical resection of an EGJ tumor can be achieved by either an esophagectomy or gastrectomy.

Type I tumors should probably be resected with an esophagectomy due to the higher risk of mediastinal lymph nodes involvement and the impossibility to achieve adequate margins with a gastrectomy. Type III tumors are adequately treated with a gastrectomy and abdominal lymphadenectomy.

Conflicting data exist regarding the optimal approach and the extent of lymphadenectomy for type II tumors. Although both approaches have shown similar oncological and clinical outcomes in these patients, we prefer an esophagectomy in order to obtain safe proximal margins and achieve adequate mediastinal lymphadenectomy.

Overall, tumor extension, lymph node involvement in preoperative imaging, patient's comorbidities and frailty, and experience of the surgical team should all be considered when deciding the surgical approach.

Table 1 describes potential advantages and disadvantages of the "esophageal" and "gastric" approach for the treatment of EGJ tumors.

NEOADJUVANT THERAPY

The optimal systemic therapy for EGJ tumor is also a debatable topic. It is clear that neoadjuvant therapy is required for locally advanced EGJ tumors to increase overall survival[40]. For this purpose, neoadjuvant chemoradiation and perioperative chemotherapy are both valid treatment modalities. However, which is the best approach for patients with EGJ tumors remains controversial.

Neoadjuvant chemoradiation

In 2012, the benefits of neoadjuvant therapy in patients with esophageal cancer were revealed by the results of the CROSS trial. This study randomized patients with esophageal or esophagogastric junction tumors to surgery alone (n = 188) or preoperative chemoradiotherapy (carboplatin and paclitaxel + concurrent radiotherapy) followed by surgery (n = 178). Patients receiving preoperative chemoradiotherapy had higher rates of R0 resections (92% *vs* 69%; $P < 0.001$) and better overall survival (49.4 mo *vs* 24 mo). In addition, 29% of the patients with chemoradiotherapy had complete pathological response [40]. The long-term results of the trial confirmed the benefits of neoadjuvant chemoradiotherapy. It is worth to mention, however, that patients with squamous cell carcinoma (ESCC) (23% of the included patients in the trial) had greater overall survival benefit than patients with adenocarcinoma[41].

Perioperative chemotherapy

In 2006, the MAGIC trial evaluated the role of perioperative chemotherapy for patients with gastric and EGJ tumors, comparing those receiving 3 cycles of Epirubicin – Cisplatin - Fluorouracil (ECF) before and after the operation against those undergoing surgery alone. The study showed significantly improved

Table 1 Potential advantages (+) and disadvantages (-) of total gastrectomy and esophagectomy for the treatment of esophagogastric junction tumors

Gastrectomy	Esophagectomy
+ Only abdominal approach, avoiding thoracotomy/thoracoscopic associated morbidity	+ Better proximal and circumferential resection margins
+ Adequate abdominal lymph node dissection	+ Extensive mediastinal lymph node dissection
+ No GERD/No PPI	+ Preservation of ¾ of stomach
- Inadequate mediastinal lymph node dissection	- Abdominal and thoracic approach
- Shorter proximal margins	- Hiatal herniation risk
- Vitamin B12 malabsorption	- Gastroesophageal reflux (necessity of PPI)
- Dumping	- Pylorospasm

GERD: Gastroesophageal reflux disease; PPI: Proton pump inhibitor.

overall and progression-free survival in patients receiving chemotherapy[42]. Two things, however, should be highlighted: only 11% of the patients had EGJ adenocarcinoma, and only 42% were able to complete the full six-cycle regimen.

In 2011, the ACCORD-07 trial compared patients receiving 2 or 3 cycles of cisplatin and fluorouracil before and after surgery with patients undergoing surgery alone. In this study, 64% of the patients had EGJ tumors. The trial showed better overall survival (38% *vs* 24%), 5-year disease-free survival (34% *vs* 19%), and higher rates of R0 resections in patients receiving perioperative chemotherapy[43].

In 2019, the FLOT trial (fluorouracil, leucovorin, oxaliplatin, and docetaxel) compared the use of perioperative FLOT (n = 356) or ECF (n = 360) plus surgery in patients with locally advanced gastric and EGJ tumors. The study demonstrated an overall survival benefit with the use of FLOT (50 *vs* 35 mo). Remarkably, only 50% of the patients completed the entire perioperative FLOT treatment[44]. These results have motivated the adoption of FLOT as the standard perioperative chemotherapy for patients with EGJ tumors.

CROSS vs FLOT

Few studies have compared the efficacy of both approaches. A propensity score-matched analysis of patients with esophageal and EGJ adenocarcinoma compared the outcomes of CROSS (n = 40) against FLOT (n = 40). Patient receiving CROSS had higher rates of complete pathological response (97% *vs* 85%; P = 0.049) and higher rates of negative lymph node metastases (68% *vs* 40%; P = 0.014). However, overall survival was similar in both groups[45]. A recent study using the National Cancer database investigated whether preoperative chemoradiation offers an advantage over chemotherapy alone in patients with lower esophageal or gastric cardia adenocarcinoma. The authors found that although patients undergoing chemoradiation had higher rates of complete pathological response (2.7 times), overall survival was similar with both treatment modalities[46]. Similar survival outcomes with CROSS and FLOT were also seen in other studies[47-49].

Expert commentary

Current evidence is weak and scarce but shows that patients with locally advanced EGJ tumors have similar survival with either preoperative chemoradiation or perioperative chemotherapy. We believe that both location and burden of disease (*i.e.* ability to obtain R0 resection) are key determinants.

For patients with Siewert type III tumors, perioperative chemotherapy is undoubtedly more reasonable due to the multiple trials supporting this approach (*i.e.* MAGIC, ACCORD, and FLOT). FLOT has shown to be the most effective regimen, and thereby should be chosen whenever possible.

Although for patients with Siewert type I and II the debate is still open, we think that avoiding the morbidity of radiation (whenever possible) is a better strategy. Patients with squamous cell carcinoma of the distal esophagus might still benefit from neoadjuvant chemoradiation. EGJ adenocarcinomas are probably better treated with perioperative chemotherapy.

Future directions: Immunotherapy

Overall survival of patients with locally advanced EGJ tumors remains low. Moreover, recurrence rates after neoadjuvant chemoradiotherapy plus surgery are high, especially among patients who do not have a pathological complete response[50-52]. Therefore, there is special interest in developing novel treatment modalities to improve outcomes. Multiples targeted therapies and immunotherapies are currently being investigated. Immunotherapy utilizes monoclonal antibodies directed against immune checkpoints proteins (*e.g.*, PD-1, PD-L1, CTLA-4). Multiples trials have shown clinical benefits with the use of immunotherapy in patients with metastatic or recurrent esophageal cancer[53-56]. The

KEYNOTE-590 study showed that adding pembrolizumab to cisplatin-fluoropyrimidine chemotherapy improved overall survival in patients with ESCC[53]. The ATTRACTION-3 trial, which included patients who had a previously treated advanced gastroesophageal cancer, showed a 2.5-mo difference in median overall survival in favor of nivolumab in comparison with chemotherapy[54]. The ATTRACTION-4 trial, on the other hand, did not show overall survival benefit, despite improvements in progression-free survival[55]. Recently, the Checkmate-577 phase III trial was conducted to compare postoperative nivolumab monotherapy against placebo in patients with locally advanced tumors who underwent resection and did not achieve complete pathologic response. Nivolumab monotherapy improved significantly disease-free survival in compared with placebo (median disease-free survival: 22.4 mo *vs* 11.0 mo; $P = 0.0003$). Interestingly, in the subgroup analysis according to histopathological type, the median disease-free survival period of patients with ESCC treated with nivolumab was better than for EAC patients. Despite this encouraging data, the trial was discontinued because of adverse events[56]. The trials PALACE-1 and PERFECT have also investigated the use of neoadjuvant chemoradiotherapy combined with immunotherapy in an effort to achieve higher rates of complete pathologic response[57,58]. However, phase 3 trials evaluating immunotherapy as neoadjuvant therapy are still warranted.

Overall, although immunotherapy has shown promising results, additional studies are needed to define safety and efficacy of this novel treatment modality.

CONCLUSION

Management of patients with EGJ tumors is challenging. Several surgical approaches and systemic therapies are currently available to treat these patients. Appropriate surgical margins and adequate lymphadenectomy should be the main goals of surgical treatment. Patients with locally advanced disease should also receive preoperative chemoradiation or perioperative chemotherapy. Tumor size and extension, nodal involvement in preoperative imaging and patient's comorbidities should all be considered for choosing the optimal treatment in these patients.

FOOTNOTES

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REVIEW

Intestinal Wnt in the transition from physiology to oncology

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Abstract

Adult stem cells are necessary for self-renewal tissues and regeneration after damage. Especially in the intestine, which self-renews every few days, they play a key role in tissue homeostasis. Therefore, complex regulatory mechanisms are needed to prevent hyperproliferation, which can lead in the worst case to carcinogenesis or under-activation of stem cells, which can result in dysfunctional epithelial. One main regulatory signaling pathway is the Wnt/β-catenin signaling pathway. It is a highly conserved pathway, with β-catenin, a transcription factor, as target protein. Translocation of β-catenin from cytoplasm to nucleus activates the transcription of numerous genes involved in regulating stem cell pluripotency, proliferation, cell differentiation and regulation of cell death. This review presents a brief overview of the Wnt/β-catenin signaling pathway, the regulatory mechanism of this pathway and its role in intestinal homeostasis. Additionally, this review highlights the molecular mechanisms and the histomorphological features of Wnt hyperactivation. Furthermore, the central role of the Wnt signaling pathway in intestinal carcinogenesis as well as its clinical relevance in colorectal carcinoma are discussed.

Key Words: Wnt signaling; Beta-catenin; Intestine; Colorectal cancer; Cell signaling; Intestinal stem cells

Core Tip: Wnt signaling pathway is a key regulator of intestinal stem cells. Mutations in this pathway are frequently found in adenomas and carcinomas of the colorectum. Therefore, it represents a potential target for anticancer therapy. This review sums up the physiological role and the regulatory mechanism of Wnt signaling in the human intestine, and moreover, discusses the central role of the Wnt signaling pathway in intestinal carcinogenesis, the morphological features associated with Wnt hyperactivation and clinical relevance of Wnt in the colorectal carcinoma.

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INTRODUCTION

The gastrointestinal epithelia are tissues that self-renew every few days. Therefore, pluripotent stem cells are needed, which have the potential to develop into different epithelial cells. These highly complex mechanisms need complex fine-tuning. An overactivation of pluripotent stem cells could lead to hyperproliferation and in the worst case to cancer development. Conversely, under-activation could lead to insufficient development of the epithelia with dysfunction of the epithelia. One main regulatory signaling responsible for intestinal epithelial development is Wnt signaling.

Since 1976 it has been known that the Wingless (*WNT*) gene in Drosophila not only influences development, but also provokes abnormalities of the mesothorax[1]. In recent decades, other genes of the Wnt family have been found and the signaling pathways around Wnt in humans have also become more and more clear. Today 19 *WNT* genes in humans are known and the Wnt pathway is known to play a critical role in embryonic development and tissue homeostasis[2]. An imbalance in Wnt signaling can lead to several diseases including carcinogenesis, neurodegenerative, metabolic and cardiovascular diseases[3]. In addition to the canonical Wnt/β-catenin pathway, which is the main focus of this review, there is also the noncanonical pathway and the noncanonical Wnt/calcium pathway[4].

This work focuses on the regulation and the role of the canonical Wnt/β-catenin signaling pathway in physiological epithelial differentiation and the molecular activities of Wnt contributing to autonomous hyperproliferation and injured cell death as hallmarks of carcinogenesis.

WNT/β-CATENIN SIGNALING PATHWAY

The most common Wnt pathway and evolutionarily conserved pathway is the canonical Wnt/β-catenin signaling (Figure 1). It consists of the transmembrane complex (Lrp5/6 and Frizzled), a destruction complex [Axin, Adenomatous polyposis coli (APC), glycogen synthase kinase-3 (GSK3), casein kinase 1 (CK1), protein phosphatase 2A (PP2A)] and β-catenin[5-7]. In the absence of the Wnt ligand, β-catenin is phosphorylated by the kinases CK1 and GSK3[8]. The phosphorylation leads to the ubiquitination and degradation of β-catenin. If Wnt binds to the transmembrane complex, the protein Disheveled is activated and turns down the destruction complex, resulting in accumulation of β-catenin in the cytoplasm[9,10]. Then, β-catenin is translocated into the nucleus and acts there as a transcription factor together with P300, B-cell CLL/lymphoma 9, pygo and T-cell factor/lymphoid enhancer-binding factor (TCF/LEF) as cofactors[11-13]. Moreover, there are inhibitors of this pathway like Dickkopf 1 (Dkk1), which binds to Lrp5 and inhibits the binding of Wnt at the transmembrane complex[14,15].

The role of Wnt/β-catenin signaling in the development of the gastrointestinal tract becomes clear when we look at the main genes which are regulated by the Wnt signaling pathway. Nuclear β-catenin activates genes which code for proteins involved in important pathways as well as processes including embryogenesis, proliferation, cell differentiation and the regulation of cell death (Table 1)[16-18].

THE NECESSITY OF WNT SIGNALING IN INTESTINAL MUCOSAL PHYSIOLOGY

In the intestinal tract, the canonical Wnt is an essential and fundamental molecular cascade to establish and constitute the mucosal barrier. However, in the different segments of the intestinal tract, the Wnt shows different cellular and molecular players as well as facets that are characteristic for each compartment. Wnt signaling is required in all parts for stem cell renewal, while Wnt overactivation in the stomach can lead to intestinal shift. Mutations in the Wnt ligands affect all parts of the intestine[19, 20]. These points are addressed further in the following paragraphs.

Table 1 Selection of assumed target genes of β-catenin

Gene	Function of the protein	Ref.
ATOH1	Transcription factor, secretory cell line differentiation	[137,138]
AXIN2	Part of destruction complex Wnt signaling	[139]
BCL2	Antiapoptotic	[140]
BIRC5	Apoptosis inhibitor	[141]
BMP4	Possible Wnt inhibitor	[142]
CCND1	Cell proliferation	[143]
CDKN2A	Cell cycle inhibitor	[144]
CDX1	Transcription factor, intestinal cell differentiation	[145]
CDX2	Transcription factor, intestinal cell differentiation	[146]
DKK1/4	Inhibitor of Wnt signaling	[147,148]
EPHB2/3	Migration and proliferation in intestine epithelial	[149]
HD5/6	Defensine, microbial defense	[150]
HEF1	Supports activation of oncogenic signaling pathways	[151]
HES1	Regulation of Notch signaling	[152]
JAG1	Ligand of Notch signaling	[153]
JUN	Cell cycle progression, apoptosis inhibitor	[154,155]
LGR5	Part of Wnt signaling	[156]
MDR1	Plasma membrane protein involved in the drug resistance	[123,124]
MET	Differentiation of intestinal epithelium	[157]
MYC	Protooncogene	[158]
MYCBP	Control of transcriptional activity of c-MYC	[159]
NOTCH2	Notch receptor	[160]
SGK1	Inhibits pro-apoptotic transcription factors	[161]
SOX9	Paneth cell differentiation	[32,162]
YAP	Transcription factor (Hippo signaling) activates genes involved in cell proliferation, suppresses apoptotic genes	[163]

ATOH1: Atonal BHLH transcription factor 1; BCL2: B-cell lymphoma 2 ; BIRC5: Baculoviral IAP repeat containing 5; BMP4: Bone morphogenetic protein 4; CCND1: Cyclin D1; CDKN2A: Cyclin dependent kinase inhibitor 2A; CDX1: Caudal type homeobox 1; CDX2: Caudal type homeobox 2; DKK: Dickkopf; EPHB2/3: EPH receptor B2/3; HD5/6: Human alpha defensin 5/6; HEF1: Human enhancer of filamentation 1; HES1: Hairy and enhancer of split-1; JAG1: Jagged Canonical Notch Ligand 1; JUN: C-Jun N-terminal kinase; LGR5: Leucine-rich repeat-containing G-protein coupled receptor 5; MDR1: Multidrug-Resistance-1; MET: Tyrosine-protein kinase Met; MYC: Myc proto-oncogene, bHLH transcription factor; MYCBP: MYC binding protein; NOTCH2: Notch Receptor 2; SGK1: Serum/glucocorticoid regulated kinase 1; SOX9: SRY-Box transcription factor 9; YAP: Yes-associated protein.

Stomach

The stomach can be divided, based on its local glands, into two main parts: The corpus/fundus and the antrum. The corpus and fundus contain oxyntic glands with chief cells, parietal cells and endocrine cells, while the antrum glands mainly contain mucous and endocrine cells[21]. Wnt/β-catenin signaling was required for the development of the embryonic fundus and in the β-catenin-deficient epithelium, parietal cells were absent[22]. In the antrum glands, $Lgr5^+$ and $Axin2^+$ stem cells were found[23]. Both proteins are regulated throughout Wnt signaling. Wnts are necessary for the maintenance of $Lgr5^+$ cells and are necessary for the zymogenic cell line from $Lgr5^+$ cells[24]. Moreover, they suppress the differentiation along the pit cell lineage. The Wnt ligands in the stomach will be secreted by pericyte-like stromal cells[25]. These cells are conserved and exist in the colon as well as in the stomach. Besides, activation of Wnt signaling in the stomach can lead to an intestinal fate in the stomach. Therefore, the mesenchymal transcription factor Barx1 represses the Wnt signaling and inhibits an intestinal shift of the stomach epithelium[26].

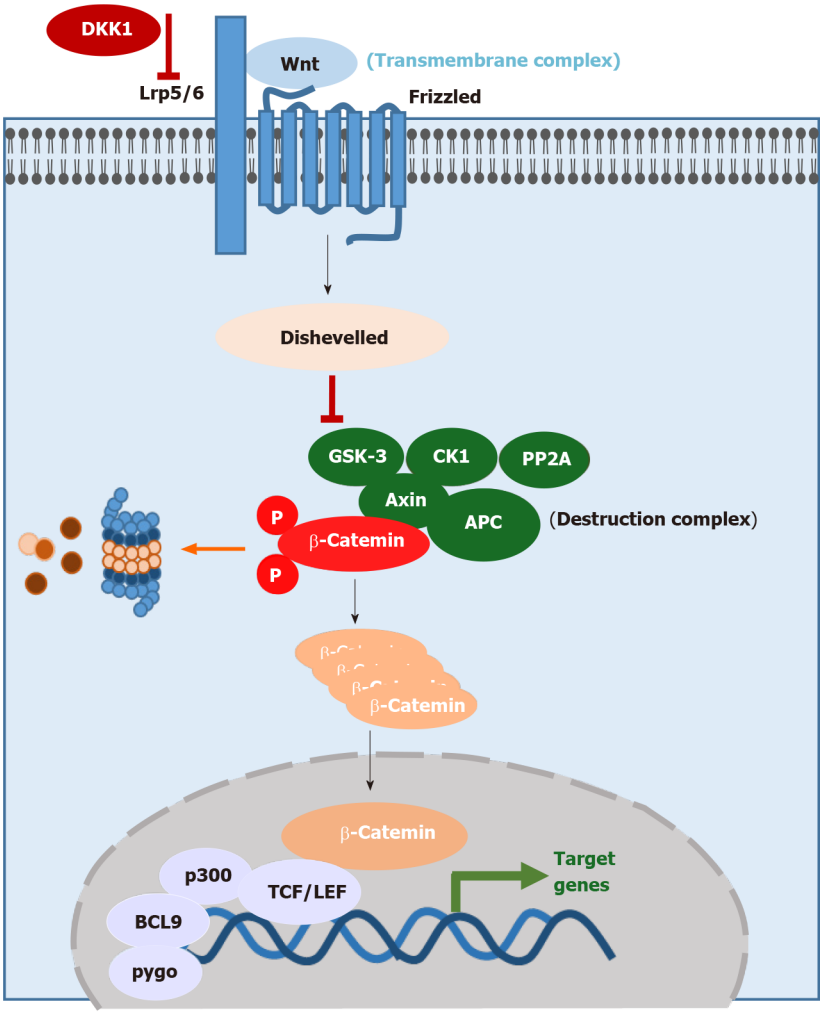

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Figure 1 Wnt signaling pathway. Activated Wnt signaling pathway: Wnt ligand binds to the transmembrane complex and activates Disheveled, which turns down the destruction complex. β-catenin accumulates in the cytoplasm and translocates in the nucleus, where it acts with several cofactors as a transcription factor. Inactivated Wnt signaling pathway: β-catenin is phosphorylated by the destruction complex and gets degraded. Dkk1: Dickkopf 1; GSK-3: Glycogen synthase kinase-3; APC: Adenomatous polyposis coli; PP2A: Protein phosphatase 2A; TCF/LEF: T-cell factor/lymphoid enhancer-binding factor; BCL9: B-cell lymphoma 9.

Small intestine

The small intestine consists of finger-like villi with an absorptive function and crypts of Lieberkühn (Figure 2). In the crypts, two different populations of intestinal stem cells (ISC) are located[27]. At the bottom of the crypts are columnar ISCs which express Lgr5, have a high division rate and are preferred for the renewal of the intestinal epithelia[28]. These cells can be activated throughout Wnt. On the other hand, there are quiescent ISCs that have a slow division rate, are less vulnerable to radiation and Wnt signaling is not activated. These cells are located above the Paneth cells and are also called +4 cells[29]. The role of these cells has not been fully investigated yet. But in the absence of columnar ISCs, quiescent ISCs can be activated and assume the tasks of columnar ISCs[30]. The localization of the subpopulation of ISC in the crypt is controlled by the surrounding mesenchymal cells through bone morphogenetic protein (BMP) signaling[27]. The regulation of the ISC occurs through Wnt3A which is secreted by Paneth cells[31].

Paneth cells are located in the base of the crypt of the small intestine next to Lgr5^{+} cells. Their differentiation is induced by SOX9, a transcriptional target and a critical regulator of Wnt signaling[32]. In contrast to other differentiated intestine cells, they do not migrate upwards to the top of the villus tip and their lifetime is, at 30 d, much longer[33]. Their main role is to synthesize and secrete defensins, anti-microbial peptides and trophic factors. Nevertheless, they seem to have an impact on crypt homeostasis.

Above the Paneth cells and stem cells is the transit-amplifying zone. The progenitor cells of the differentiated enterocytes are settled here, which can divide themselves two to five times[34,35]. All differentiated cells with the exception of Paneth cells migrate from the crypts upwards to the villi. The main parts of differentiated cells are enterocytes, which make up 80%-90% and have an absorptive function. In addition to them, there are tuft cells, goblet cells, enteroendocrine cells and microfold cells that are

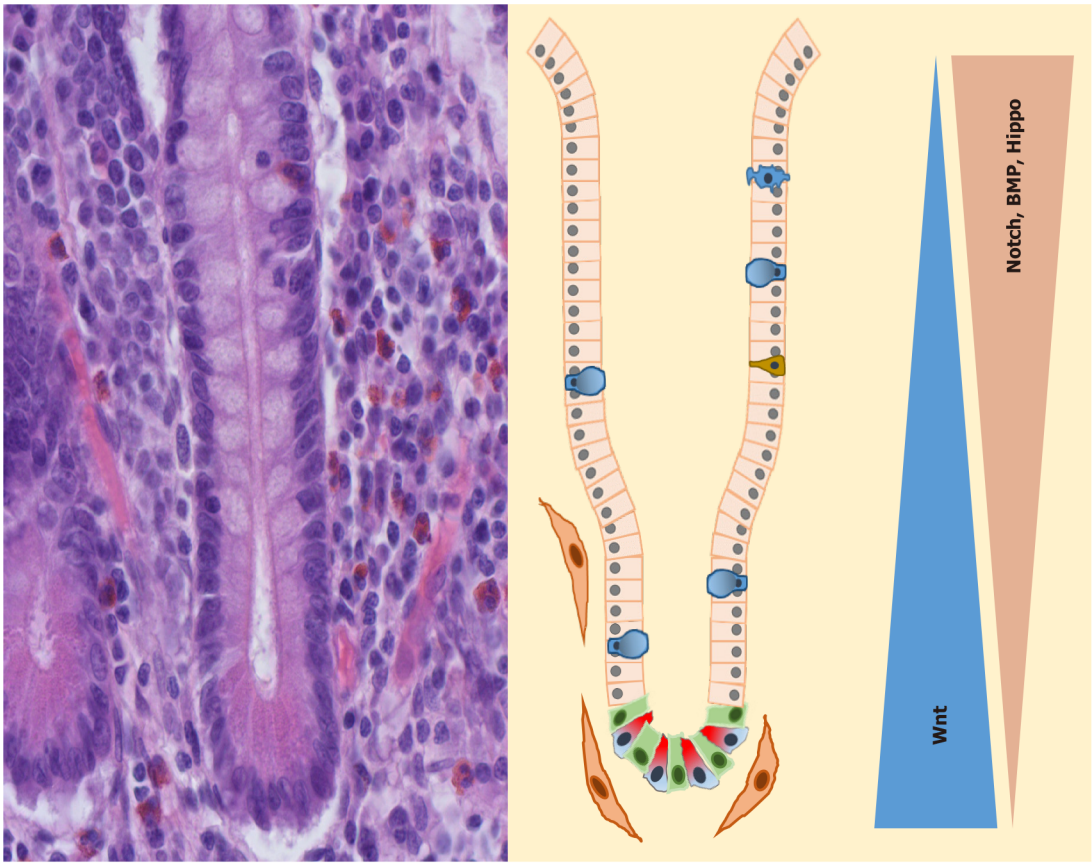

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Figure 2 Small intestinal crypt of Lieberkühn with signaling pathway gradients. On the left sight histology of a small intestinal crypt (400 × Hematoxylin eosin) and on the right a schematic drawing of a small intestinal crypt with intestinal stem cells (green), Paneth cells (red), goblet cells (light blue), tuft cell (blue) and neuroendocrine cell (yellow). BMP: Bone morphogenetic protein.

also termed M cells[35,36].

That Wnt signaling is essential for intestinal development has been already shown in the work of Pinto *et al*[37]. Overexpression of the Wnt inhibitor Dkk1 leads to a loss of crypts and reduced epithelial proliferation[37]. Furthermore, inhibition of Dkk leads to a reduced rate of fission of crypts in postnatal growth[38]. A negative autoregulatory feedback loop of Wnt signaling prevents a hyperactivation of Wnt signaling[28,39].

Colon

The colon has, in contrast to the small intestine, crypts, but no villi. The so-called colonocytes are functionally equivalent to the enterocytes[35]. Like the small intestine, the colon epithelia renew themselves through crypt-based columnar ISCs[35]. The work of Davies *et al*[40] revealed that Wnt activity is lower in the colon than in the small intestine. This may be influenced by the fact that instead of Paneth cells the colon epithelia have deep secretory cells with similar functions to Paneth cells, but in contrast to Paneth cells, they do not secrete Wnt ligands[35,41]. Furthermore, *in vitro* studies show that the reaction of Wnt-signaling activation also differs between the left and the right colon[42]. In embryonic development, a Wnt3A gradient plays an important role in hindgut extension and colon formation[43]. Like the small intestine, the colon epithelia include goblet cells, tuft cells and enteroendocrine cells[35].

THE COMPLEX REGULATION NETWORK OF WNT SIGNALING

As mentioned above, the Wnt signaling pathway is a highly conserved pathway and essential for intestinal homeostasis. To preserve this homeostasis, precise fine-tuning is absolutely necessary. The regulation of Wnt ligands occurs on different pathway levels. The mechanisms involved in this regulation are explained below and summed up in Figure 3.

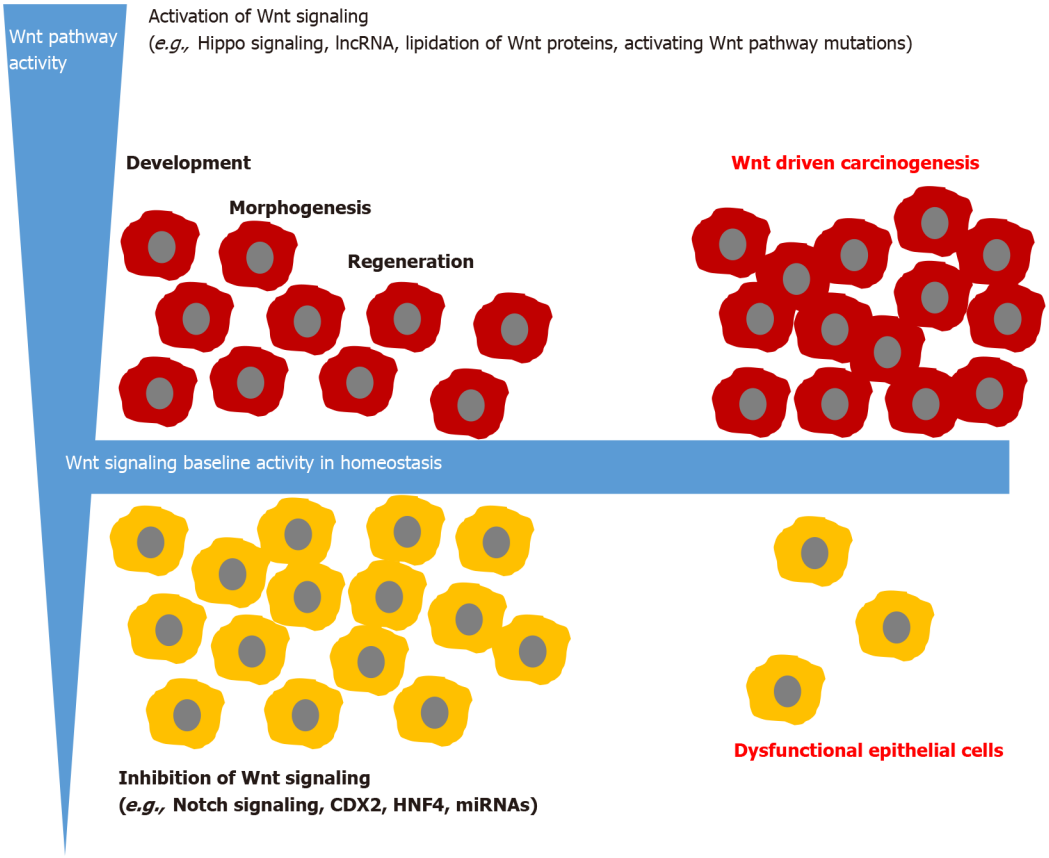

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Figure 3 Wnt signaling regulatory mechanisms in intestinal cell development. Wnt signaling balances intestinal development, morphogenesis and regeneration due to a gradient of Wnt pathway activity in epithelial layers with major activated cells (red) and minor activated cells (yellow). In Wnt-driven carcinogenesis, the gradient of Wnt pathway activity is lost and major activated, neoplastic cells (red) dominate. lncRNA: Long non-coding RNA; miRNAs: MicroRNAs.

Notch signaling pathway

Notch signaling is one of the most important signaling pathways in terms of adjacent cellular communication and regulation of gastrointestinal stem cells[44]. It plays a crucial role in determining whether a cell develops into a secretory or an absorptive cell[44]. Deletion of NOTCH1 and NOTCH2 leads to hyperplasia of secretory cells[45]. It is not surprising that Wnt and Notch signaling act closely together and regulate each other[46,47]. The amount of Notch correlates here inversely with the amount of β-catenin[48,49]. On the other hand, Disheveled, which is part of the Wnt signaling, inhibits Notch signaling[50,51]. As Notch signaling requires cell-cell contact, Paneth cells are important for controlling the Notch signaling of small ISC[52]. In conclusion, Notch signaling determines cell fate to absorptive cell lines, while Wnt signaling drives cells to secretory cell lines[35,53].

Caudal-related homeobox transcription factor 2

Caudal-related homeobox transcription factor 2 (CDX2) is essential for human development. In the gastrointestinal tract, it determines gastric and intestinal development[54]. In adult mice, the absence of CDX2 leads to a cessation of intestinal differentiation[54]. In various works it has been shown that CDX2 activates Axin 2, which is part of the destruction complex in Wnt/β-catenin signaling[55,56]. Yu *et al*[56] showed in their work that CDX2 upregulates not only Axin 2 but also GSK-3β, which is also part of the destruction complex. The absence of CDX2, which in colorectal cancer is directly correlated with a higher tumor grade, leads to an activation of Wnt signaling[57].

BMPs

BMPs belong to the transforming growth factor-β (TGF-β) family. They are produced by mesenchymal cells especially at the tip of the villus and generate a contrary gradient with Wnt through the crypt-villus axis[58]. At the crypt base, BMP signaling is repressed by BMP inhibitors like gremlin and chordin-like 1 secreted by smooth muscle cells or myofibroblasts[59]. BMP represses ISC proliferation, while the influence of BMP on Wnt signaling is the subject of controversial debate. The work of He *et al*[60] postulates that BMP inhibits Wnt signaling, while the work of Qi *et al*[61] describes a direct suppression of $Lgr5^+$ cells through BMP without changes in the Wnt target genes.

Hippo signaling pathway

Hippo signaling is a highly conserved pathway and important for intestinal homeostasis and regeneration. Inactivation of Hippo signaling leads to an activation of the transcription factor Yes-associated protein 1 (YAP1), which has the highest activity at the bottom of the crypts[62]. YAP1 is an oncogene that is a facultative regulator of stem cell homeostasis and an essential regulator for the regeneration of the intestinal epithelial after injury[62]. Hippo and Wnt signaling are closely linked to each other[63]. YAP1 increases the transcriptional activity of β-catenin, while active Hippo signaling leads to the formation of the destruction complex of Wnt signaling[64,65].

Hepatocyte nuclear factor 4

Hepatocyte nuclear factor 4 (HNF4) is a transcription factor family that mainly regulates metabolism in cells. Especially fatty acids have a high impact on ISC homeostasis[66]. Chen *et al*[67] show in *in vitro* studies that HNF4α and HNF4γ activate genes involved in fatty acid oxidation and that HNF4 is necessary for stem cell renewal in the intestine. Studies about the interaction of HNF4 and Wnt are rare, few studies indicate that HNF4 may regulate Wnt signaling. The study by Yao *et al*[68] demonstrated that HNF4α is downregulated in human colon carcinoma and showed in *in vitro* experiments that HNF4α suppresses Wnt/β-catenin signaling. These results coincide with the data shown in hepatocellular carcinoma[69].

Posttranslational modification of Wnt ligands

Wnt ligands need posttranslational modifications before they can activate Wnt signaling. In the endoplasmic reticulum, Wnt ligands were glycosylated and lipidated[70]. These modifications are essential for intracellular transport, secretion of Wnt ligands and signaling[71,72].

Wnt signaling could also be inhibited by posttranslational palmitoylation. Acyl-CoA synthetase 5 (ACSL5), a mitochondrial enzyme, activates long-chain fatty acids, while binding a thioester. ACSL5-dependent palmitoylation of Wnt2β leads to an accumulation of Wnt2β in the mitochondrion and a decrease in Wnt signaling activity[73].

Furthermore, the degradation of Wnt components by the proteasome can be regulated *via* ubiquitination through ligases. For example a phosphor switch in the E3 ubiquitin ligase RNF43 leads to a lack of degradation of Frizzled and therefore to Wnt activation[74]. The ligase RNF43 itself is inhibited by receptor Lgr4[75]. Park *et al*[76] summed up the different regulation possibilities of Wnt signaling throughout ubiquitination and deubiquitination. The ubiquitination is done by E3 Ligases while deubiquitination is done by deubiquitinating enzymes. In Wnt signaling, every protein component is targeted by ubiquitination or deubiquitination[76]. Therefore, it is an important regulator of Wnt signaling.

Non-coding RNAs

Long non-coding RNAs are over 200 nt long non-coding RNA molecules. As reviewed in Zarkou *et al*[77], they can act as a Wnt enhancer by transcriptional activation of genes coding for Wnt proteins or by interaction with transcription factors regulating Wnt signaling.

MicroRNAs (miRNAs) are small 18-25 nt long non-coding RNA molecules and can bind on their target messenger-RNA (mRNA) and suppress translation. Rahmani *et al*[78] summed up about 17 miRNAs that target mRNAs encoding for proteins of Wnt signaling. Here, they can act as an activator of Wnt signaling by suppressing translation of mRNA encoding for the destruction complex or as a suppressor of Wnt signaling, by inhibiting translation of mRNAs encoding for transmembrane complex or β-catenin. Kim *et al*[79] examined the crosstalk between stress-driven ribosome dysfunction and Wnt signaling. A proteinkinase R-activating ribosomal insult leads to changes in the Wnt and connective tissue growth factor crosstalk, which leads to progression in cancer stemness.

Other pathways

Despite the above-described pathways, growing evidence demonstrates that other pathways including the mitogen-activated protein kinase (MAPK) pathway, TGF-β signaling, and phosphoinositide 3-kinase/protein kinase B (PI3K/AKT) pathways involved in cell proliferation and survival have an influence on Wnt signaling[80]. It is reported that MAPK signaling regulates Wnt activity on stemness phenotypes in colorectal carcinoma cells[80,81]. Moreover, it has been found that Wnt and TGF-β pathways interact with each other to regulate genes participating in epithelial to mesenchymal transition (EMT)[82]. Hu *et al*[83] depict that epidermal growth factor receptor mediated PI3K/AKT activation enhances Wnt signaling activity through promoting β-catenin translocation, leading to increased tumor cell invasiveness.

HYPERACTIVATION OF WNT SIGNALING DRIVES PATHOPHYSIOLOGY

In spite of these regulatory mechanisms, Wnt hyperactivation is not always avoidable. In this context,

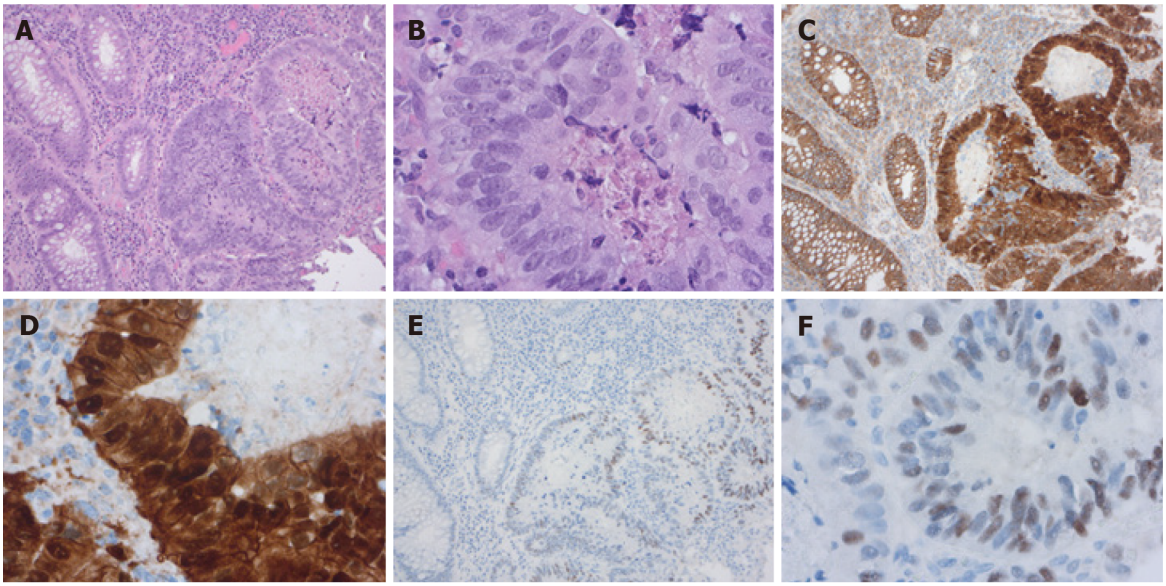

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Figure 4 Colorectal carcinoma. A: Invasive growth and loss of polarity [100 × Hematoxylin eosin (HE)]; B: Cellular atypies (400 × HE); C: β-catenin staining (100 ×) membranous in normal epithelial, nuclear staining in dysplastic cells; D: β-catenin staining (400 ×) with partly extensive accumulation of β-catenin in the nucleus; E: Positive staining of c-myc (a target of β-catenin) in the dysplastic cells (100 ×); F: Positive nuclear staining of c-myc (400 ×).

controlled activation must be distinguished from autonomous activation. Controlled activation is triggered by a stimulus outside the cell and determined through the presence of the stimulus, while autonomous activation is mainly triggered through modifications of proteins involved in the pathway and independently of the regulatory mechanism. The detailed mechanisms which lead to hyper-activation of Wnt signaling and the histomorphological correlation will be discussed hereafter.

Molecular mechanisms resulting in Wnt hyperactivation

As mentioned above, Wnt signaling is a complex regulated signaling pathway and many possibilities lead to hyperactivation of Wnt signaling in the intestine. Especially Wnt activation, while the loss of *APC* gene is well-studied *in vitro* and *in vivo*. In Drosophila, *APC* loss induced intestinal tumorigenesis [84]. A germline mutation in the *APC* gene with a loss-of-function mutation leads to familial adenomatous polyposis, representing a hereditary disease characterized by hundreds of colorectal adenomas[85]. But hyperactivation is not always accompanied by pathological tissue growth. In intestinal epithelial after injury, Wnt is also hyperactivated and enables regeneration[86]. Nevertheless, there is a fine line between Wnt activation for tissue regeneration and tissue hyperplasia. Ahmed *et al* [87] show in mice that Wnt and Notch signaling balance transmissible murine colonic hyperplasia and colitis induced by *citrobacter rodentium*. In the chronically inflamed intestine such as bowel disease, Wnt signaling is activated[88]. These patients had an increased risk of developing dysplasia and colorectal carcinoma[89]. Abnormal β-catenin expression was more closely linked to E-cadherin alterations in inflammatory bowel disease-related cancers than in sporadic cancers suggesting that specific alterations in this pathway may differ in these two cancer groups[90].

As long as Wnt signaling is controlled by other pathways, hyperproliferation of epithelial is stoppable. Problematic is uncontrolled Wnt activation, which leads to a permanent-growth stimulus. This could be caused by loss-of-function mutations in the genes encoding for the destruction complex. As mentioned above, familial adenomatous polyposis is a good example of this. But growth stimulation alone is not sufficient for carcinoma development. Fearon and Vogelstein generate the model of the adenoma-carcinoma-sequence[91]. They postulate that stepwise genetic alterations in oncogenes and tumor suppressor genes lead to hyperproliferative epithelial, low-grade and high-grade adenoma to carcinoma development. Besides *APC* mutations, which are hypothesized as a key event in adenoma development, gain-of-function mutations in *KRAS* and loss of functions in *P16-INK4*, *TP53* and *Smad4* are described in the model of multiple step carcinogenesis[92]. It is assumed that this model applies to 80% of colorectal carcinoma[93]. Nonetheless, not only *APC* mutations but also mutations in *KRAS* influence Wnt/β-catenin signaling[84]. In cell culture, KRAS stabilizes β-catenin through inhibition of GSK-3β, while others postulate that KRAS mutations activate Wnt signaling through DNA demethylation[93,94]. Interestingly, *APC* mutation and Wnt activation is a common finding in colorectal cancer, but not in carcinoma of the small intestine, even though Wnt activity in the small intestine is higher than in the colon[40,95]. That suggests that in colorectal carcinogenesis the Wnt activation is not triggered by a regulatory activation of Wnt signaling, but through an autonomous, uncontrolled activation of the Wnt signaling pathway.

In the stomach, bile acid reflux leads to an epigenetic downregulation of Dkk1, an inhibitor of Wnt signaling[96]. The bile acid-induced downregulation of Dkk1 is correlated with gastric intestinal metaplasia and might be triggered by Wnt activation. Other studies have demonstrated high expression of Dkk1 in gastric carcinomas[97].

Morphological changes caused by mutations associated with Wnt activation

The genotypic changes in colorectal adenomas lead to phenotypic changes (Figure 4). Adenoma with the classical adenoma-carcinoma-sequence often present macroscopically or endoscopically as polypoid lesions, while tumors with CpG island hypermethylation and *BRAF* mutations often present as flat mucosal lesions[92]. *APC* mutations are more often in adenomas with villous or tubulovillous formation, which are reminiscent of small intestinal villi, but *APC* mutation is also found in tubular adenomas which had elongated crypts[98]. Furthermore, Paneth cell metaplasia is also a common finding in conventional adenoma, following the adenoma-carcinoma-sequence. Joo *et al*[99] examined colonic epithelial neoplasms for Paneth cell metaplasia and Paneth cells were found in 38.5% of the conventional adenoma. This Paneth cell metaplasia was always associated with positive nuclear β-catenin staining[99]. The adenoma cells also show, depending on their grading, enlarged, hyperchromatic nuclei and loss of polarity and decreased numbers of goblet and absorptive cell lines[100]. In conclusion, hyperactivation of Wnt in the colon shifts the phenotype to a small intestinal-like phenotype.

As in the intestine, APC downregulation occurs in gastric adenomas[101]. In the stomach, the downregulation of APC is mostly caused by hypermethylation of the APC promoter and might be triggered by *Helicobacter pylori* infection[102]. Koushyar *et al*[103] summed up the parts of Wnt signaling which are deregulated in gastric cancer. In gastric cancer organoids, Wnt inactivation leads to a shift from morphological poorly carcinoma not other specified to signet-ring cell carcinoma[104].

CLINICAL RELEVANCE OF WNT ACTIVATION IN THE INTESTINE

Clinical relevance of Wnt activation in gastric cancer

In studies, Wnt signaling was upregulated in more than 80% of the examined gastric cancers and may mark Lgr5 stem cells[105]. The detailed mechanism which leads to Wnt activation is similar to colorectal cancer and is reviewed in detail by Chiurillo[106]. Mao *et al*[107] examined that Wnt1 overexpression accelerated the growth of gastric cancer. Wnt/β-catenin signaling inhibitors suppress gastric tumor growth in a mice model[108].

Clinical relevance of Wnt activation in the small intestine

Chen *et al*[109] showed cells of the Paneth cell lineage are present in intestinal adenomas. They secrete Wnt 3 and a deletion of Paneth cells leads to reduced growth of adenomas in the small intestine in APC^{min} mice. The authors concluded that Wnt3 is required for early tumorigenesis in the small bowel.

Clinical relevance of Wnt activation in colorectal cancer

In recent decades, the role of genetic aberration as a prognostic value has moved increasingly to the fore. It is therefore evident that APC mutations, which occur in the majority of microsatellite stable colorectal cancers, are examined to determine whether they had a prognostic value of colorectal cancer. Jorissen *et al*[110] analyzed over seven hundred patients with sporadic colorectal cancer and found that wild-type *APC* correlates with poor prognosis (5-year survival) in microsatellite stable proximal colon cancer. On the other hand, some studies indicate that nuclear β-catenin promotes metastasis of colon cancer, which usually display poor prognosis, by EMT[111,112].

As mentioned above, mutations that activate Wnt/β-catenin signaling are common genetic events in colorectal cancer and usually occur in an early state of carcinogenesis. Therefore, Wnt inactivation is a possible target for preventing tumor progression and as a potential treatment of colorectal cancer. 5-aminosalicylic acid (5-ASA) is a well-established treatment against inflammatory bowel disease, especially in ulcerative colitis. Therefore, it has not only anti-inflammatory but also anti-proliferative effects[113]. Several cohort studies and case-control studies have demonstrated that 5-ASA treatment is associated with a reduced colorectal cancer risk in patients with ulcerative colitis[114-116]. Therefore, guidelines recommend 5-ASA treatment for ulcerative colitis patients also under the aspect of cancer prevention. The anti-proliferative effect is forced by PP2A-dependent accumulation of nuclear β-catenin [117]. Munding *et al*[118] examined the role of the chemopreventive effects of 5-ASA *in vivo*. After three years, there were no significant differences regarding the progression of adenomas between the patients treated with 5-ASA and the placebo group. But in the group treated with 5-ASA, a significant decrease in nuclear β-catenin expression was found[118]. Further studies with a longer treatment time were necessary because the development of carcinoma through the adenoma-carcinoma sequence takes about ten to fifteen years[119]. Serafino *et al*[120] examined in their study the β-catenin expression and the expression of the β-catenin regulated proteins c-Myc and Cyclin D1 in bowel disease and found elevated

Table 2 Selection of potential target opportunities to inhibit Wnt/β-catenin signaling

Target	Effect	Ref.
Ligand-dependent Wnt signaling activation		
Wnt ligands	Wnt inhibitors	[164]
	Posttranslational modification	[165]
Dkk1	Stabilization, increase of Dkk1	[128,166]
Transmembrane complex	Inhibition of Lgr5/6	[167]
	Inhibition of Frizzled	[168,169]
Dishevelled	Inhibition	[170]
Ligand independent Wnt signaling activation		
Destruction complex	Stabilization of the destruction complex	[171,172]
β-catenin	Increase of degradation	[130,131]
	Inhibition of translocation to the nucleolus	[173]
β-catenin cofactors		[174]
Ribosome biogenesis		[134]
Oncolytic viruses		[132,133]

Dkk1: Dickkopf 1.

expression levels of these proteins especially in low-grade and high-grade dysplasia. These results emphasize the potential benefit of Wnt signaling inactivation as a predictive cancer therapy.

As reviewed by Zhu *et al*[121], Wnt activation has an impact on the resistance to chemotherapy in colorectal adenocarcinoma. Hu *et al*[122] determined that Wnt activation through exosomal Wnt secretion of fibroblasts leads to an increase in chemoresistance of cancer stem cells. Zhang *et al*[123] also identified the tumor microenvironment as a crucial factor in Wnt-induced chemoresistance. The increased chemoresistance in Wnt upregulated cancers is not only caused by enhancing the expression of antiapoptotic proteins, but also by enhancing the expression of multidrug resistance proteins[123, 124]. Zhong *et al*[125] summarized different studies where chemoresistance is associated with Wnt activation in conventional radiochemotherapy, but also in targeted and immunotherapy. Wnt signaling seems to have a big impact on the response to cancer therapy. Hence, the development of a personalized therapy targeting components of the Wnt signaling pathway in treatment of cancer is required.

WNT/β-CATENIN SIGNALING AS A POTENTIAL TARGET IN THE PREVENTION AND TREATMENT OF INTESTINAL CANCER

Application of Wnt inhibitors might be a possible therapeutic strategy to inactivate the Wnt pathway in cancer, for example obviation of binding of Wnt to Frizzled, stabilization of Dkk or destruction complex, inhibition of the transmembrane complex or Disheveled, application of β-catenin antagonist and antagonist of β-catenin cofactors, *etc*. Different drugs targeting Wnt pathway are currently in clinical trials, as reviewed in detail in Caspi *et al*[126]. Kleeman *et al*[127] postulate that there may be a difference in the therapeutic approach in ligand-dependent and ligand-independent tumors. Therefore, the localization of the mutation should be taken into account in the choice of Wnt signaling-targeting therapy. Ligand-dependent tumors should be targeted to the ligands or the transmembrane complex. In ligand-independent tumors, such as APC mutated tumors, targeting transmembrane complex is useless. A therapeutic option in these tumors is increased degradation of β-catenin. This is achieved by a stabilization of the destruction complex or directly by an increase of β-catenin degradation. One way to stabilize the destruction complex is an increased polymerization of conductin/axin2[128]. *In vitro* it represses the growth of colorectal cancer cells[128]. An opportunity to strengthen the degradation of β-catenin is *via* the proteasome through binding of molecules, which induces proteolysis. Kessler *et al*[129] examined potential binding sites of β-catenin proteolysis targeting chimeras (PROTACs). The first PROTACs are tested in mice and showed, in $APC^{min/+}$ mice, prevention and regression of colorectal cancer[130]. The E3 Ligase, TRIM58 enhances β-catenin degradation in gastric cancer and is a potential therapeutic target[131]. A different approach would be oncolytic viruses. *In vitro* and in a mice model, the adenovirus CD55-Smad4 represses tumor proliferation in metastasis by, inter alia, suppression of

Wnt signaling[132]. Adenoviruses that inhibit tumor growth by repressing the Wnt pathway have also been developed for other carcinomas such as hepatocellular carcinoma[133]. Another possible therapeutic approach in Wnt-activated tumors would be the inhibition of the ribosome biogenesis. Raveux *et al*[134] show that ribosome biogenesis dysfunction alleviates Wnt-driven tumor initiation and reduces cancer cell proliferation. In a study, kinase inhibitors in gastric cancer were screened for Wnt pathway inhibition and 34 kinases inhibit Wnt signaling more than 50%[135]. Potential targets to inhibit Wnt/β-catenin signaling are summarized in Table 2.

However, it must be noted that there could be a YAP/TAZ-dependent transcriptional reprogramming which leads to a lineage reversion and a Wnt-independent tumor growth, which can lead to failure of Wnt signaling inhibitors[136].

Development of therapeutic approaches by targeting Wnt signaling main players is challenging though it brings new hope for the management of colorectal cancer in the future.

CONCLUSION

The Wnt/β-catenin signaling pathway is a highly regulated pathway and essential for intestinal homeostasis. Disruption of this homeostasis with Wnt signaling hyperactivation can lead to tumor development and indeed Wnt activation is common in human colorectal cancer. The prognostic value of Wnt activation in colorectal cancer has not been fully elucidated yet. Furthermore, components of the Wnt signaling pathway have been brought into focus as possible targets in anti-cancer therapy and as possible adjuvant treatment for chemoresistant cancers.

FOOTNOTES

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REVIEW

From *Helicobacter pylori* infection to gastric cancer: Current evidence on the immune response

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Abstract

Gastric cancer (GC) is the result of a multifactorial process whose main components are infection by *Helicobacter pylori* (*H. pylori*), bacterial virulence factors, host immune response and environmental factors. The development of the neoplastic microenvironment also depends on genetic and epigenetic changes in oncogenes and tumor suppressor genes, which results in deregulation of cell signaling pathways and apoptosis process. This review summarizes the main aspects of the pathogenesis of GC and the immune response involved in chronic inflammation generated by *H. pylori*.

Key Words: Gastric cancer; *Helicobacter pylori*; Chronic inflammation; Host immune response

Core Tip: Understanding the factors related to the host, infection by *Helicobacter pylori* and the mechanisms of tumor evasion are fundamental to understand the development of gastric cancer (GC). However, in the face of a complex immune environment, there are still many questions to be answered. Thus, we highlight in this work the main aspects related to GC, from infection and gastric microenvironment to immune response.

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INTRODUCTION

Helicobacter pylori (*H. pylori*) infection is acquired mainly in early childhood and if not treated properly can remain for life. Because of this, the infection is highly present around the world and has been linked to a wide spectrum of gastrointestinal diseases[1]. The prevalence of the bacteria varies according to geographic regions, age of the patient, socioeconomic status, education, living environment and profession. In developing countries, such as those in Latin America, they can have a prevalence of up to 80% of infected adults[2]. Recent work by Elzouki *et al*[3] evaluated 114 patients with gastric cancer (GC) and indicated that the infection rate per *H. pylori* was 63.2%. Through its virulence factors, it damages the gastric mucosa and the hormonal release, changing the stomach environment, often asymptomatically[4].

Thus, after a chronic inflammatory process, the host may develop GC in the long term, with adenocarcinoma as the most common type. GC is a worldwide public health problem since approximately one million new people are diagnosed each year with this pathology. Among the types of neoplasms, gastric adenocarcinoma is the fifth most common and is the third cause of cancer-related death worldwide. Although there is a strong association between *H. pylori* infection and gastric neoplasms, only 1%-3% of those infected end up developing GC[5]. Also in this sense, in 1994, the World Health Organization identified *H. pylori* as a group 1 carcinogen, which means a certain relationship to carcinogenesis, confirming the role of this bacterium in the process of GC development.

The mechanisms involved in this process are complex and not well known, but it is known that the tumor evasion mechanism of the immune response has a fundamental role in this process[5]. The development of this neoplastic microenvironment results from genetic and epigenetic changes in oncogenes and tumor suppressor genes, which results in deregulation of cell signaling pathways and the process of apoptosis[6]. It is known that the prognosis of patients with GC is not good, with an average 5-year survival rate of less than 20%[7]. The prognosis of patients with GC can also vary according to their classification, which can be based from anatomical location to recent molecular discoveries.

This work aims to provide an updated review on the main characteristics that lead to the development of gastric neoplasms from gastric infection by *H. pylori* in order to provide solid data that help in the knowledge about GC.

VIRULENCE FACTORS OF *H. PYLORI*

H. pylori has a lot of virulence factors that favor its maintenance in the hostile gastric environment. The recognition of these factors can even determine how serious the infection with the bacteria can be. Here, the main factors will be listed.

Flagella

Of primary importance for the pathogenic action of *H. pylori*, the flagella play a crucial role in colonization by this pathogen[8]. About four to eight flagella make up the *H. pylori* flagellar group, and each of these unitary flagella is made up of three structures: the basal body, the hook and the filament [9]. Such composition allows this bacterium not only the motility through gastrointestinal fluids, known as "swimming" but also in solid or semi-solid media, known as "spreading" and "swarming" movements, which are crucial for entry into gastrointestinal epithelial cells[8].

Chemotaxis

In addition, still in the mobility and fixation of this bacterium in the gastric environment, the chemotaxis process guarantees the interaction with molecules such as mucins, sodium bicarbonate, urea and sodium chloride, facilitating the effectiveness of the infection[10,11]. Another factor of paramount importance in the role of chemotaxis occurs in the process of continuity of infection, causing it to become chronic. In this sense, the chemotaxis ability allows *H. pylori* to circumvent the host's immune responses, achieving chronic infection[12].

Adhesion molecules

Various adhesion molecules are described as important for the colonization process by *H. pylori*, in addition to helping to protect this pathogen against mucin activity and contributing to access to important nutrients, such as nickel, which are essential for effective infection[13]. An important adherence factor that has been described in the literature is BabA, responsible for the connection with Lewis H-1 type antigens. In addition to this adhesion function, this molecule also appears to be related to the type of clinical manifestation presented by the host in the face of infection[14-16].

Another molecule of importance for the adhesion of this pathogen to gastric tissue is the outer inflammatory protein, which is also linked to the production of interleukin (IL)-8, mucosal damage and duodenal ulcer[17]. In addition, studies also argue that there is a possible relationship between outer inflammatory protein expression and greater chances of developing GC[18]. Still on the adhesion molecules of this pathogen, 33 proteins form the *H. pylori* outer membrane proteins (Hop)[19,20]. Even though most of them still do not have their activity well described or understood, some of them already have their role in the pathogenicity of *H. pylori* highlighted, such as BabA (HopS), SabA (HopP), HopQ and HopZ. BabA is related to the specific link to the b and H-1 Lewis antigens from the surface of the gastric epithelial cells, and SabA is associated with binding to Le^x and the adherence of the bacterium to laminin[21-23]. Meanwhile, the HopQ and HopZ proteins are relatively consolidated as to their importance for pathogen adherence, and the former also appears to be related to gene A associated with cytotoxins (*CagA*) gene expression[24].

Pathogenicity

Several molecules are listed as important for the pathogenesis of *H. pylori*. Among them, the role of urease stands out, which is activated even before the bacterium adheres to the gastric tissue, making an adequate acclimation of the pH of the gastric environment, regulating it to protect this bacterium[25]. In addition, urease is related to the production process of ammonia derived from urea, due to the urea channels that allow the entry of this substance in the pathogen and the intrabacterial action of this enzyme[26]. Furthermore, in addition to its role in colonization, urease seems to be important for regulating the immune response, controlling a macrophage-pathogen interaction, modulating the pH of the phagosome and ensuring the survival of *H. pylori*[27].

One of the proteins most expressed by *H. pylori* is the catalase that converts hydrogen peroxide into water and molecular oxygen[28]. Of paramount importance for the protection of the pathogen against the host's immune responses, prevention of death mediated by the complement system and avoidance against the action of phagocytes, catalase seems to be related to the clinic of gastric tumors and cancers [29,30]. Apparently, this process occurs through chronic inflammation, prevention of apoptosis and induction of mutagenesis (processes related to the action of this enzyme)[31].

H. pylori strains can be classified as *CagA* positive or *CagA* negative. Apparently, *CagA* is the main virulence factor of this pathogen, and its greatest expression seems to be directly related to more aggressive clinical manifestations, such as acute gastritis, gastric ulcer and GC[32-35]. This process is related to its ability to affect cellular motility, proliferation and apoptosis, affecting the entire conformation of gastric tissue and predisposing inflammatory pathways that facilitate these clinical presentations[35]. Still on the *CagA* gene, different forms of phosphorylation that occur (EPIYA A, B, C or D) are related to different results, with types C and D (Western and Eastern strains) more related to the outcome of GC[36].

Vacuolating cytotoxin A is an essential cytotoxin for the pathogenesis of *H. pylori*, promoting autophagic processes during the acute phase of infection, in addition to promoting the appearance of impaired autophagosomes and unbalancing cell proliferation and death during a chronic phase of infection[30]. Present in all strains of this pathogen, vacuolating cytotoxin A can be encoded by different genopatterns, being the strains s1 and m1 more related to higher levels of inflammation and consequently less indolent clinical presentations, such as gastric atrophy and carcinoma[37-40].

Another determinant factor in the pathogenesis of *H. pylori* is the *cag*-pathogenicity island, which is composed of approximately 32 genes[41]. The *cag*-pathogenicity island is responsible for the encoding of a type 4 secretion system that helps modulate the cellular metabolism of the host cell, translocate virulence factors such as CagA to the gastric epithelial cells and upregulate proinflammatory cytokine secretion[42,43]. Also, strains that present *cag*-pathogenicity island are more related to peptic ulcer and GC[44,45]. Moreover, the interaction of CagA with the SH2 containing protein tyrosine phosphatase-2 is extremely relevant. The CagA/SH2 containing protein tyrosine phosphatase-2 link happens through a tyrosine phosphorylation-dependent process, which promotes activation of the SH2 containing protein tyrosine phosphatase-2/extracellular signal-regulated kinase/mitogen-activated protein kinase pathway and consequently causes cytoskeleton alterations known as the "hummingbird" phenotype[46, 47]. These changes interfere in cellular growth and motility, which may predispose the host to genetic mutations and further GC[48]. In addition, *H. pylori* lipopolysaccharides also play an important role in the pathogenesis of this bacterium. The lipopolysaccharides are capable of binding laminin and as a consequence promote a gastric leakiness and further cellular apoptosis[49]. Furthermore, *H. pylori* lipopolysaccharides might be related to the development of GC, given that it upregulates toll-like receptor 4 and enhances cell proliferation, both *via* activation of the mitogen-activated protein kinase

kinase 1/2-extracellular signal-regulated kinase 1/2-mitogen-activated protein kinase pathway[50].

Still in the virulence factors of *H. pylori*, several others can be mentioned, such as heat shock proteins, superoxide dismutase and degrading enzymes (proteases and phospholipases), being listed in this article.

HOST IMMUNE RESPONSE TO INFECTION

H. pylori induces a significant immune response in the gastric environment of infected individuals. The onset of the inflammatory processes related to the infection occurs with the promotion of innate immunity mechanisms, involving the triggering of pattern recognition receptors of gastric epithelial cells by bacterial components such as lipopolysaccharide, NapA and nucleic acids[51]. The aforementioned recognition of foreign antigens by immune system receptors leads to the activation of intracellular signaling pathways that culminate in the release of proinflammatory cytokines, which promote the activation and recruitment of CD4+ and CD8+ T cells to the gastric environment[52]. Subsequently, a chronic inflammation against *H. pylori* infection is established, being characterized by a polarization of T helper (Th) 1/Th17 responses, which is followed by the action of regulatory T (Treg) cells responsible for controlling the inflammatory process.

The inflammatory pattern varies between groups of patients and seems to be strongly influenced by age[53]. Figure 1 summarizes the main changes in the immune response to infection by *H. pylori* according to age. In general, a predominance of the Th1 response is commonly observed in adults, along with high levels of interferon γ (IFN-γ), tumor necrosis factor α, IL-1β and IL-8[54-56], which is mostly responsible for the recruitment of neutrophils and further setup of an inflammatory environment[57]. However, when it comes to children, this pattern of cytokine release and consequent responses are not presented in the same way as adults. In a previous investigation with *H. pylori*-positive adults and children evaluating Treg and Th17 responses in the gastric mucosa, our group observed that children have higher expression of Treg-related cytokines such as IL-10 and transforming growth factor beta 1 (TGF-β1) than adults. On the other hand, adults had a prominent expression of cytokines associated with Th17 responses (IL-1β, IL-17A and IL-23) compared to children. Moreover, that study found that the expression of FoxP3+ Treg cells in the gastric mucosa was significantly higher in infants than in adults, and more intense infiltration of mononuclear and polymorphonuclear cells was observed in the latter group[58]. In another investigation evaluating the levels of cytokines associated with innate and Th1 responses in the gastric mucosa of infected individuals, we demonstrated that children express significantly higher concentrations of tumor necrosis factor α and IL-1α than adults, whereas the contrary was observed with regard to the expression of IL-2, IL-12p70 and IFN-γ. In addition, a progressive reduction in the levels of IFN-γ and IL-12p70 was observed with aging among adults, including elderly individuals, whereas a similar process was observed with the expression of IL-1, IL-2, IL-12p70 and IFN-γ in children[59].

H. pylori-induced Th1 responses have been associated with the development of corpus gastritis, which can result in gastric atrophy and intestinal metaplasia, important in precancerous lesions[60]. Moreover, Treg cells have been associated with various relevant protumor mechanisms in the setting of GC. Enhanced tumor infiltration of FoxP3+ Treg cells have been positively correlated with poor outcomes among patients with gastric adenocarcinoma[61].

Although the aforementioned immune profiles play important roles in the GC onset and progression, growing evidence have emphasized the remarkable protumoral activities associated with Th17 cells. High levels of IL-17 in the tumor environment have been related to increased concentrations of vascular endothelial growth factor and enhanced tumor vascularization. In addition, cytokines promote IL-6 production in tumor cells, and it is a protein that induces vascular endothelial growth factor release as well but also stimulates STAT3, which suppresses apoptosis and prolongs the survival of malignant cells[62]. In a recent study enrolling patients with *H. pylori*-related diseases, our group demonstrated that GC patients lack IL-27 production both in the gastric environment and peripheral blood[63]. This cytokine is an important inhibitor of Th17 responses by impairing the expression of RORγT, the main IL-17A transcription factor[64].

GC CLASSIFICATION

The classification of GC can be useful for determining a more effective diagnosis as well as a more targeted treatment and better prognosis for cancer patients. The classification system can use anatomical location, degree of invasion, lymphatic involvement, histological type and molecular subtypes[65,66]. Among several classification options, there are older ones that have fallen out of use or continue to be used today (such as Lauren's), and there are recent updates from renowned institutions such as the World Health Organization.

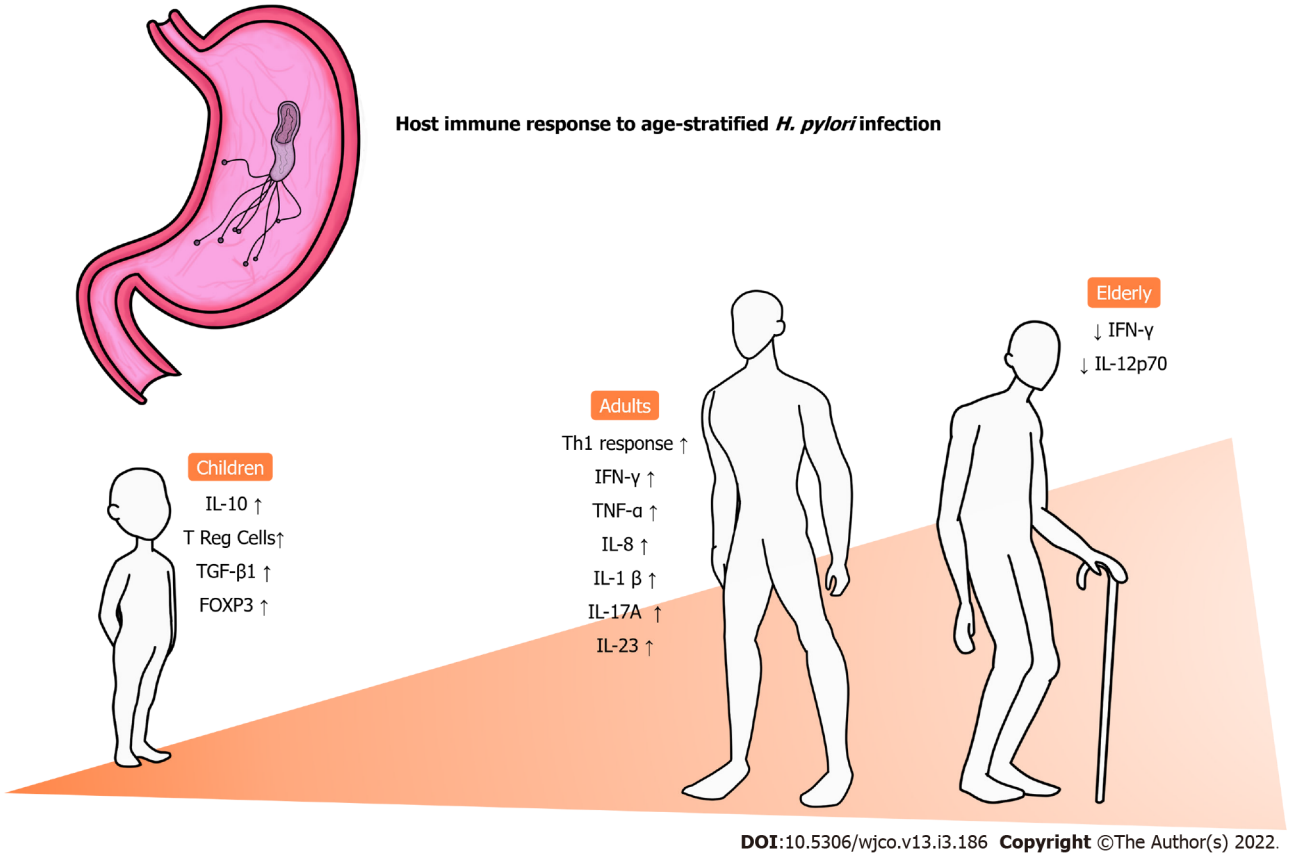

Figure 1 Host immune response to age-stratified *Helicobacter pylori* infection. *H. pylori*: *Helicobacter pylori*; IL: Interleukin; TNF-α: Tumor necrosis factor α; IFN-γ: Interferon γ; T Reg Cells: Regulatory T cells.

The anatomical classification can be divided into: (1) Cardial; and (2) Distal. The location of tumors of origin at the gastroesophageal junction, whether esophageal or gastric, may not be identified until the tumor has already reached an expressive size. The literature has shown that the tumor originating in the cardia usually presents a more aggressive behavior in relation to the distal ones, frequently invading the gastric walls[67]. In addition, the occurrence of tumors in the distal region has decreased to the detriment of those in the proximal region[68].

Classification according to the degree of invasion can be done in early or advanced cancer. The early type, limited to the mucosa and submucosa, has a lower degree of development and injury and has a 5-year survival rate of 85% to 90%, while patients with the advanced type have a 5% to 20% survival rate. Furthermore, the advanced type can be evaluated by the Borrmann classification: polypoid (type 1), ulcerated with defined edges (type 2), ulcerated with ill-defined edges (type 3) and plastic linitis, characterized by diffuse infiltrate without evidence of ulceration (type 4)[69].

Lauren's Histological Classification, widely used since its publication in 1965, has been useful in the discussion of GC. This classification divides gastric adenocarcinoma into two histomorphologic types, intestinal (well, moderately or poorly differentiated) and diffuse (undifferentiated, with or without signet ring cells). The intestinal type is more common in males and older patients, with a better prognosis. It is characterized by tumor cells that unite and organize into glandular formations, just as it occurs in intestinal adenocarcinomas. In addition, the intestinal type usually develops in an environment of atrophic gastritis and presents greater expression of the e-cadherin adhesion molecule [70]. The diffuse type, more prevalent in young individuals and more easily identified in early stages, is characterized by tumor cells that invade neighboring tissues, with little cohesion, loss of e-cadherin expression, without gland formation and with marked fibrosis. In addition, this type presents endocrine markers more frequently and has a higher production of basic fibroblast growth factor[71-73].

Among the most recent classifications, in 2019 the World Health Organization updated the classification of tumors of the digestive system, including GC. In this new approach, histogenesis and the degree of differentiation were not considered, but it recognizes several types of malignant epithelial tumors (tubular, papillary, poorly cohesive signet ring phenotype, another type of poorly cohesive, mucinous, mixed cell) as well as rare variants[74].

The National Institute of Health Cancer Genome Atlas project helped to redefine the molecular classification of GC into four subtypes: (1) GC with Epstein-Barr virus infection; (2) Microsatellite unstable tumors; (3) Genetically stable tumors; and (4) Chromosomally unstable GCs[75,76]. The first constitutes about 9% of GCs and is more common in males, has lesions in the bottom and gastric body with a lower

mortality rate and has hypermethylated DNA[77]. The second type represents 22% of GC cases and has a high mutation rate (with high frequency in the KRAS pathway), generally related to an epigenetic event[75,78]. The third type is usually aneuploid and diagnosed early, representing about 20% of GC cases, in addition to having a predominance of diffuse histology and located in the distal region of the stomach[75]. The latter type represents 50% of CG and has histology of the intestinal type, and its frequency is high in cancers of the esophagogastric junction. Chromosomal instability is the result of DNA aneuploidy and mutations in various proto-oncogenes and tumor suppressor genes[75,79].

PATHOGENESIS

Precancerous lesions

Atrophic gastritis: In atrophic gastritis (AG), there is an inflammatory process that promotes gland loss and decreased secretory function, modifying the gastric environment, which may be associated with a state of achlorhydria or hypochlorhydria[80]. A recent study showed that the relative risk for GC was 1.7 in moderate AG and 4.9 in severe AG compared to none or mild AG (control)[81]. However, it seems to be possible to identify the evolution of this risk early. In the study by Miki *et al*[82], it was reported that the progression of AG is closely linked with progressive reductions in the levels of pepsinogen I and II. Therefore, measuring these levels can be an opportunity to assess the progression of gastritis [82]. Another way to assess the risk of progression is through the location and extent of atrophy. A staging system based on the degree of atrophy and the topography of atrophy, called Operative Link for Gastritis Assessment was created for this purpose. In this system, stages 3 and 4 are strongly associated with GC development[83].

Gastric intestinal metaplasia: This lesion is characterized by the replacement of the gastric epithelium by two types of intestinal epithelium. This replacement is generally considered a condition that predisposes to malignancy and an increased risk for GC, especially type III (incomplete)[84,85]. Although the presence of this lesion is considered by many authors as a mild form of dysplasia[86], it is still controversial whether gastric intestinal metaplasia is really a precancerous lesion. After all, several studies have shown that gastric intestinal metaplasia is not always seen in patients who progress to GC [87]. However, further studies are needed to define this question.

Along with this questioning, it remains uncertain whether the eradication of *H. pylori* promotes the improvement of these precancerous lesions, as there are recent works that have not found any change [88]. However, most studies that assess patients for more than 5 years after the eradication of the bacteria, demonstrate improvements in these lesions. Some possible reasons for these discrepancies are ethnic variations, disease stage, follow-up period, medications used and resistance to the drugs used[89, 90].

GC MICROENVIRONMENT

The GC microenvironment has a complex local immune response, and factors that promote the growth and expansion of cancer cells can be observed. Although the microenvironment is still poorly elucidated, some components have already been recognized. Inflammatory cells, fibroblasts and macrophages associated with cancer, endothelial cells and other infiltrating immune components play an important role in this process[91,92] (Figure 2).

Cancer-associated fibroblasts

Cancer-associated fibroblasts are involved in the synthesis and remodeling of the extracellular matrix and are directly related to angiogenesis, mechanical factors of the tumor and metastatic modulation[93]. The secretome of these cells produce TGF-β, FGF5 and specific growth arrest protein 6 that contribute to the proliferation and invasion of cancer cells[94]. In addition, the presence of vascular endothelial growth factor, IL-6 and chemokine ligand (CXCL9) can be observed, which together with TGF-β reduce the immune response of T lymphocytes[95]. Cancer-associated fibroblasts contribute to increase the stiffness of tumor tissue, compress blood vessels generating a hypoxemic process and contribute to a more aggressive cancer, providing immune evasion and less effective therapeutic response[96,97]. Interestingly, a study using mice found that these cells contribute to the progression of GC, but their functions are not fully understood[98]. This whole process contributes to an immunosuppression in the tumor microenvironment and creates an ideal environment for the development and progression of tumor cells in the affected tissue and possibly other tissues. These cells are probably important in the process of malignant or benign evolution of GC and should be better explored so that their knowledge is directed to therapeutic contexts.

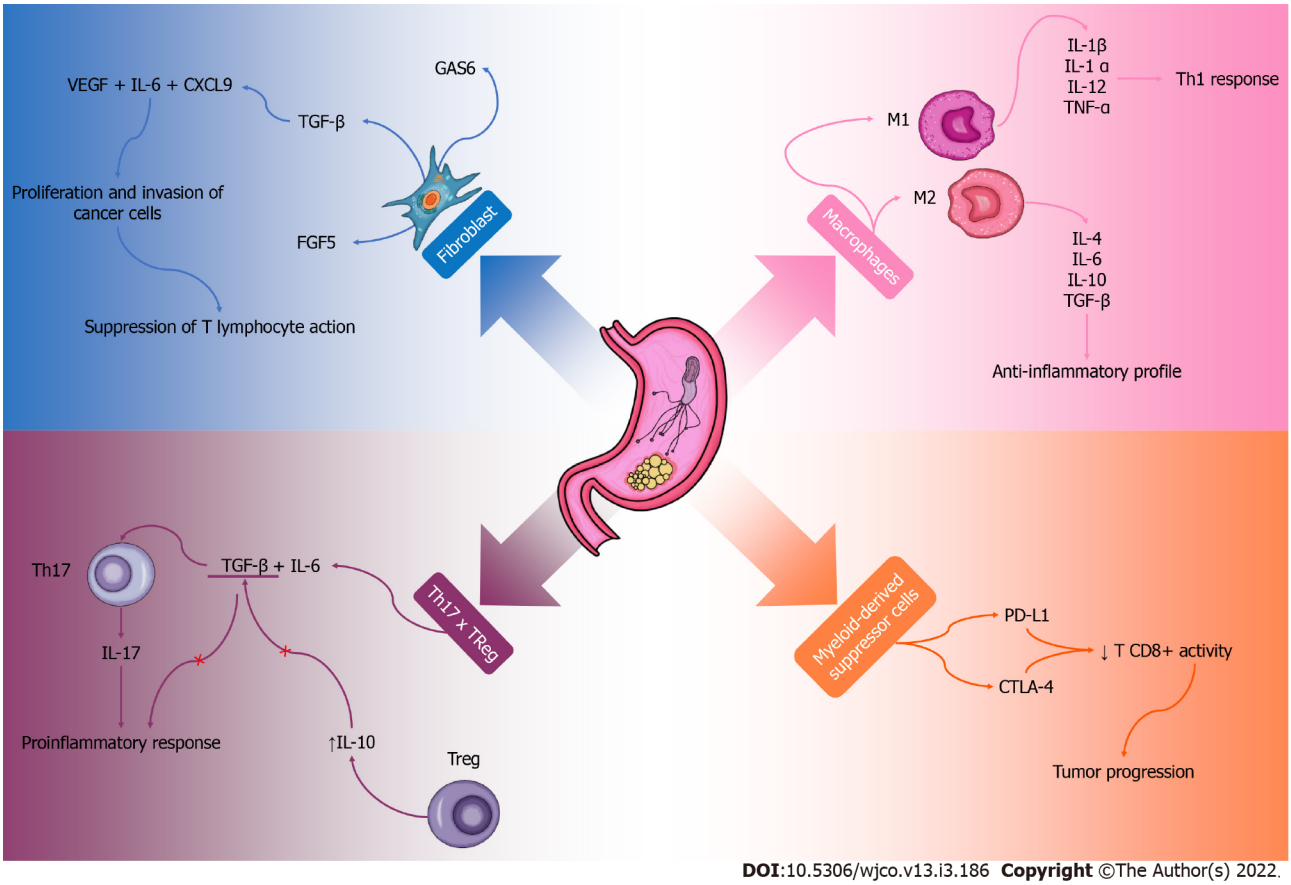

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Figure 2 Summary scheme on the microenvironment of gastric cancer. TGF-β: Transforming growth factor beta; FGF5: Fibroblast growth factor 5; GAS6: Specific growth arrest protein 6; VEGF: Vascular endothelial growth factor; CXCL9: Chemokine ligand; PD-L1: Programmed death ligand 1; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; IL: Interleukin; Th: T helper cell; TNF: Tumor necrosis factor; Treg: Regulatory T cell.

Tumor-associated macrophages

Tumor-associated macrophages are also abundant components of the immune system that play an important role in the microenvironment of GC[99,100]. They can be M1 type and produce proinflammatory cytokines such as IL-1β, IL-1α, IL-12, tumor necrosis factor α, IL-12 and CXCL9, which polarize and recruit components of a Th1 response profile[93,101]. This process culminates in the inhibition of tumor growth[102]. In contrast, type M2 stimulates a Th2 profile secreting cytokines such as IL-4, IL-6, IL-10, IL-13, IL-33, TGF-β and IL-10, which have an anti-inflammatory profile and important tumor activity[103]. They act on tumor progression, metastasis and angiogenesis, which are important factors for the tumor formation microenvironment[104]. The polarization of M1/M2 macrophages is conducted according to the inflammatory profile of the tumor microenvironment or therapeutic intervention. More studies would be interesting in order to observe possible therapies in order to reverse the polarization of M2 macrophages in M1 so that with a more proinflammatory profile, the immune system is stimulated, and the fight against the tumor is more effective.

Myeloid-derived suppressor cells

Myeloid-derived suppressor cells are part of the cell population involved in immune responses and are observed in the tumor microenvironment. They suppress the cytotoxic function of CD8+T cell activity and antitumor, as they have a high expression of programmed death ligand 1 and cytotoxic T-lymphocyte-associated protein 4[93,105]. An interesting study in mice observed that the expression of IL1-β is associated with a greater recruitment of these suppressor cells in the tumor microenvironment [106]. Baumann *et al*[107] observed that the neutralization of the activities of these cells, concomitant with the inhibition of the checkpoint, obtained a greater efficacy in cancer therapy.

Relationship between Th17 /Treg

Regarding the expression of cytokines in the microenvironment of GC, there is an important increase in Th17 and Treg cells causing an imbalance in the relationship between these cytokines. This phenomenon is observed gradually with the progression of cancer[108]. The increase in Th17 cells, stimulated by TGF-β and IL-6, promote tumor progression due to increased expression of IL-17 and consequently greater local inflammation[109]. However, Treg cells provide an immunosuppressed environment,

stimulating a high production of IL-10 and inhibiting the production of TGF-β. Thus, these Treg cells stimulate the progression of the GC, decreasing the host's immune surveillance in the tumor microenvironment[110]. Apparently, the balance of the Th17 and Treg relationship in the gastrointestinal tract reflects the integrity of the mucosal immune response and plays an important role in the mechanisms of tumor progression and metastasis.

Most studies are *in vitro* and murine models. Despite few studies on human models with GC, it is important to highlight the role of immunosuppression in the tumor microenvironment, which is essential for the development of cancer and for possible metastases. The immune response in the tumor microenvironment has a direct link to the host's general immune response to cancer. These responses are dependent on factors such as genetics, polymorphisms, chronic diseases and the use of drug therapies. In addition, the lack of studies in different stages of human life is a problem since the immunological profile is different throughout life. More studies on the microenvironment of GC are needed, so it would be possible to better understand the mechanism of the immune response and possibly find even more effective therapies in the treatment of this cancer.

TUMOR EVASION MECHANISMS TO THE IMMUNE RESPONSE OF THE HOST

During the initial development of tumors, including GC, their cells use several mechanisms to resist innate immune response and prevail in the organism, and when the tumor achieves more advanced stages it evades from the action of T effector cells[111]. Of note, the tumor microenvironment is a protagonist in that context[112]. The immunosuppression promoted by the programmed cell death protein 1 is strongly related to the immune evasion and worse outcomes in GC[113]. In that context, the podoplanina is an immune checkpoint molecule that has been associated with the immune response evasion in various malignancies[114].

Liu *et al*[115] reported in their study that high levels of podoplanina-expressing cells infiltrating gastric tumors were associated with an increased recruitment of protumoral macrophages and T effector cell dysfunction. Moreover, the infiltrating podoplanina cells contributed to the reduction of INF-γ, granzyme B and perforin-1 levels as well as with an increased expression of programmed cell death protein 1, T cell immunoglobulin and immunoglobulin and mucin-domain containing-3. These findings suggest that the expression of those cells is a tumor evasion mechanism by gastric tumor cells[115].

Another study observed that the macrophage-derived chemokine CXCL8 plays a crucial role in tumor progression in GC patients by mediating immune response evasion and metastasis. CXCL8 acts by reducing the infiltration of Ki67 CD8+ T cells and inhibiting them through the expression of programmed death ligand 1 in macrophages[116]. Interestingly, a study evaluated a GC-derived extracellular compound and concluded that it presents immunosuppressive activities through selective inhibition of CD161CD3 natural killer cells, proliferative stimulus and reduction of the intracellular levels of IFN-γ, granzyme B and perforin[117]. Complementarily, Zhang *et al*[118] investigated the importance of IL-10-related tumor-associated macrophages in the GC immune response evasion and observed that a tumor microenvironment with high levels of IL-10 tumor-associated macrophages was characterized by the infiltration of Treg cells and dysfunction of CD8+ T cells.

Shi *et al*[119] demonstrated in a study that the density of Foxp3+ Treg cells and A2aR/CD8+ T cells were highly expressed in the tumor microenvironment and were able to avoid immune responses against GC. The mechanism used by the FoxP3+ Treg cells was the cell apoptosis induction through the ATP decomposition into adenosine as well as the inhibition of CD8+ T cells through the A2aR pathway, leading to an immunosuppressive effect[119]. Notably, a study showed that IL-10-producing regulatory B cells have the potential to avoid the immune surveillance in patients with GC and predict poorer outcomes since the population of CD19CD24+hiCD27 B cells included cells that are able to suppress CD4+ T cells and the production of IFN-γ by autologous CD4+ T cells[120].

Another protein, the costimulatory molecule B7-H4, is a member of the B7 inhibitors family expressed in tumor-related monocytes and macrophages. It is considered an important component of GC immune system evasion, being that it is correlated to invasion depth and to the presence of venous and lymphatic invasion as well as to the expression of HLA-DR[121]. Interestingly, circulating tumor cells undergo an epithelial-mesenchymal transition that allows them to survive in several metastatic environments. In this sense, an assay demonstrated that patients with GC had subtypes of epithelial-mesenchymal transition markers able to regulate ULBP1 (a major member of the natural killer group 2 member D ligand family) in the circulating tumor cells, which aid in immune response evasion[122].

Considering that the tumor purity consists of the proportion of cancer cells in the tumor and is intimately related to the tumor microenvironment characteristics, it is important to emphasize that the low tumor purity implies an unfavorable prognosis, accentuated infiltration of Treg, M1 and M2 macrophages, high expression of immune checkpoints and recruitment of immunosuppressor molecules [123]. A computational study used the interface mimicry technology in order to predict host-pathogen interactions in the context of *H. pylori* infection and their repercussions in GC. This study found that the *H. pylori* infection interferes with the apoptosis of host cells through proteins such as HP0231, which is able to impair the CASP6 homodimerization, a crucial step for apoptotic signaling.

The aforementioned interaction might explain the *H. pylori*-induced resistance to death of host cells, a well-known characteristic associated with carcinogenesis[124]. The studies mentioned here highlight the importance of understanding the mechanisms that lead to the immune system evasion of GC in order to better understand the pathophysiology of the disease as well as to improve prognosis assessments and therapeutic tools for affected individuals. Finally, it is evident that the *H. pylori* virulence factors are closely related to the gastric carcinogenesis by means of adaptative mechanisms that not only contribute to the infection persistence but also unleash premalignant changes in the gastric microenvironment. These variations progress and perpetuate along with tumor development, aiding in its evasion from the immune response.

FUTURE PERSPECTIVES

Faced with such complex pathways to be understood, future work needs to detail the immune response to bacterial infection, especially to help with early intervention in patients who may develop CG. This may enhance the discovery of new pharmacological therapies that interfere with precancerous lesions and even in advanced stages of cancer. In addition, they should also help in the discovery of new, non-conventional, non-invasive, highly specific biomarkers capable of providing early detection of GC.

CONCLUSION

Infection by *H. pylori*, its relationship with the host's immune system and oncogenesis as well as the tumor evasion mechanisms is crucial for the understanding of the mechanisms involved in the appearance and progression of GC. In view of the chronic inflammation process, a variety of factors can affect the patient's prognosis, especially according to the individual's age. The gastric microenvironment has not been well established, and some fundamental components for the progression of GC have been recognized to be related to the host's immune response.

FOOTNOTES

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MINIREVIEWS

Possible relationship between refractory celiac disease and malignancies

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Abstract

Celiac disease (CeD) is a chronic autoimmune disorder that is triggered by gluten in genetically susceptible individuals, and that is characterized by CeD-specific antibodies, HLA-DQ2 and/or HLA-DQ8 haplotypes, enteropathy and different clinical pictures related to many organs. Intestinal lymphoma may develop as a result of refractory CeD. If a patient diagnosed with CeD is symptomatic despite a strict gluten-free diet for at least 12 months, and does not improve with severe villous atrophy, refractory CeD can be considered present. The second of the two types of refractory CeD has abnormal monoclonal intraepithelial lymphocytes and can be considered as pre-lymphoma, and the next picture that will emerge is enteropathy-associated T-cell lymphoma. This manuscript addresses "CeD and malignancies" through a review of current literature and guidelines.

Key Words: Refractory celiac disease; Enteropathy-associated T-cell lymphoma; Pre-lymphoma; Low grade lymphoma

Core Tip: Malignancies are among the leading consequence of celiac disease (CeD), and intestinal lymphoma and adenocarcinomas in particular. Enteropathy-associated T-cell lymphoma type 1 has been shown to develop from refractory CeD type 2, while the association of CeD with other cancer types is controversial. Decades of reported studies suggest that a non-delayed diagnosis of CeD and strict adherence to a gluten-free diet significantly reduces the rate of cancer development associated with CeD.

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INTRODUCTION

Celiac disease (CeD) is a chronic autoimmune disorder that is triggered by gluten in genetically susceptible persons with HLA-DQ2 and/or HLA-DQ8 haplotypes, and is characterized by CeD-specific antibodies and enteropathy[1-3]. The prevalence of CeD in the general population is approximately 1% on serological screening, and 0.6% as histologically confirmed[3].

CeD can affect many organs, and can cause or trigger, or be associated with different clinical pictures, including growth retardation, short stature, chronic diarrhea, constipation[1], iron deficiency anemia[4], dermatitis herpetiformis[5], dental enemal defects[6], aphthous stomatitis[7], rickets, osteoporosis[8,9], arthralgia, arthritis[10], idiopathic epilepsy[11], peripheral neuropathy[12], ataxia[13], abnormal liver tests, autoimmune hepatitis[14], type1 diabetes mellitus[15], IgA deficiency[16], psychiatric comorbidities[17], intestinal lymphoma[3], etc. It is not known exactly why these clinical pictures emerge as different manifestations in different patients, as there are complex underlying mechanisms. Although the relationship between CeD and intestinal lymphoma is known, there have been many studies and case reports suggesting its association with other malignancies. For the present manuscript, a systematic literature search of PubMed/MEDLINE was carried out using the search terms "Celiac disease AND guideline, and Celiac disease AND malignancy" and a review was made on the subject of "CeD and malignancies" in current literature and guidelines in line with the following structure: (1) Pathogenesis of CeD; (2) Refractory CeD; (3) Enteropathy-associated T-cell lymphoma (EATL); (4) CeD and malignancies; and (5) Conclusion.

PATHOGENESIS OF CELIAC DISEASE

Although the pathogenesis of CeD is not fully understood, it is considered to be attributable to the coaction of genetic, environmental and immunologic factors. The HLA-DQ2 and/or HLA-DQ8 haplotypes are necessary for CeD development. Studies have shown that around 4% of HLA-DQ2 + cases develop CeD, and that HLA-DQ2 and HLA-DQ8 negative CeD development is extremely rare [18]. It is evident that environmental factors are at the core of the CeD pathogenesis, of which gluten is the *sine qua non* trigger. The gliadin proteins found in gluten are composed of glutamine and prolamine residues, and cannot be fully digested, even in a healthy person. HLA-DQ2 and HLA-DQ8 proteins are located on the surface of intestinal antigen-presenting cells. Undigested gliadin peptides in the intestinal lumen pass through the intestinal epithelium and undergo cross-linking and deamination through tissue transglutaminase (tTG) in the lamina propria. The glutamine contained within gliadin is converted to glutamic acid, bound to HLA-DQ2 and HLA-DQ8 and presented to CD4+ T cells. The cross-linking of gliadin and tTG results in the formation of tTG antibodies that impair the function of tTG. Activated CD4+ T cells cause the production of pro-inflammatory cytokines like interferon-γ that contain T-helper cells that worsen the inflammatory effect in the process. Matrix metalloproteinases cause the degradation of the extracellular matrix and damage to the basement membranes, resulting in an increase in natural killer (NK) T lymphocytes within the epithelial cell. Gliadins also upregulate the expression of the zonulin protein by increasing intestinal permeability in both CeD patients and healthy people. Increased anti-tTG levels are also known to inhibit tTG and make gliadin harder to digest, which in turn increases tTG activity, resulting in a vicious cycle. Intraepithelial lymphocytes (IELs) include T cell receptor (TCR)αβ+ and -γδ+ T cells, and NK cells. Most of these TCR+ IELs express a variety of NK cell receptors, and in addition, the number of CD8+ TCRαβ+ and TCRγδ+ increases. Consequently, characteristic lesions of CeD develop by apoptosis[2,18-22].

CeD is in general similar to other autoimmune diseases, but has a very clear and indispensable trigger: gluten. Gluten-induced intestinal lesions and autoantibodies begin to improve in the absence of gluten. Anti-tTG antibodies increase to protect against the disease, and are at the center of the pathogenesis. They may appear before villous atrophy develops and can induce CeD[21].

REFRACTORY CeD

Refractory CeD (RCeD) patients are those with a pre-existing diagnosis of CeD whose CeD-related symptoms fail to improve, and in whom villous atrophy develops despite a strict gluten-free diet for more than 12 months[23-25]. RCeD is mostly diagnosed after the age of 50 years, but younger cases have

been identified. The incidence for both types of RCeD is in the 0.04%-1.5% range[3].

When RCeD is suspected, a second endoscopy and several biopsies are mandatory. Duodenal biopsies show Marsh type III, and sometimes Marsh type II[3]. The presence of subepithelial collagen extending to the lamina propria in the duodenal second part, chronic inflammation and crypt hypoplasia (not hyperplasia) with villous atrophy are common microscopic findings of RCeD[23].

Refractory CeD is divided histologically into two subgroups according to the immunophenotype of IELs: type 1 (RCeD-1) and type 2 (RCeD-2). RCeD-1 has a normal intraepithelial lymphocyte phenotype while RCeD2 has an abnormal clonal lymphocyte population[25]. In RCeD-1, the symptoms are less severe, and the endoscopic and histological features are similar to active uncomplicated CeD. RCeD-1 shows the same normal immunophenotype as CeD, often leading to difficulties in differential diagnosis from CeD, although differentiating between RCeD-1 and RCeD-2 is mandatory due to the different treatment strategies and prognosis[3].

The immunophenotype of abnormal IELs in RCeD-2 is different to that of RCeD-1. It has been reported that interleukin-15 and somatic mutations in JAK1 or STAT3 in the proliferation of aberrant T cells play an important role in the formation of RCeD-2[24]. Cording *et al*[26] identified a complex mutational profile of JAK1 and STAT3 that activated the NF-κB pathway in CeD-associated lymphomagenesis.

While most lymphocytes express CD3, CD8 and polyclonal TCRβ, RCeD-2 is characterized by abnormal T cells that do not express surface CD3 or CD8, but instead express intracellular CD3 by a TCR gamma rearrangement[23-25], and these cells also express NK surface markers[24,27]. RCeD-1 becomes involved when abnormal T cells account for less than 20%, and RCeD-2 for more than 20%. RCeD-2 may be referred to as pre-lymphoma or low grade lymphoma due to the high risk of conversion to EATL[3,28]. Verbeek *et al*[29] suggest that the quantification of abnormal T cells using flow cytometry is preferable to T cell clonality analyses in differentiating RCeD patients. The use of a cut-off value of 20% for the classification of patients can also support the selection of long-term follow-up and treatment.

Figure 1 summarizes the properties of RCeD-1, RCeD-2 and EATL.

The goal of treatment is to prevent RCeD-1 patients from converting to RCeD-2, and then to EATL, in that a total of 52% of RceD-2 patients have been reported to develop EATL within 4–6 years of diagnosis of RCEeD-2[30]. Immunosuppressive drugs are used together with nutritional support for the treatment of RCeD-1. Although similar therapies have been applied for RCeD-2, their usefulness is limited. In such patients, autologous hematopoietic stem cell transplantation following high-dose chemotherapy is an alternative treatment[3,31].

ENTEROPATHY-ASSOCIATED T-CELL LYMPHOMA

Enteropathy-associated T-cell lymphoma accounts for less than 1% of all non-Hodgkin lymphomas, and as such is considered a rare GI lymphoma[3]. Approximately 50% of RCeD-2 patients are thought to develop overt lymphoma within 5 years of diagnosis[18]. EATL occurs predominantly in patients in the sixth and seventh decades, and usually develops in those diagnosed with CeD[25,32,33]. EATL is thought to be derived from IELs, and the abnormal immune phenotype of IELs seen in RCeD-2 indicates early-stage lymphoma development. To date, two histologically subtypes of EATL have been described [23].

A microscopic examination of type I EATL (EATL-1) reveals transmural infiltration including pleomorphic medium- to large-size neoplastic lymphocytes, histiocytes and eosinophils. Mitotic figures and necrosis are common, and enteropathic changes such as villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis may be seen in the non-tumor gastrointestinal tract mucosa[25,33]. Tumor cells in EATL-1 have a pattern of CD2+, CD3+, CD5-, CD4-, CD7+, CD8-, CD56-, TCR- (usually), CD103+ and CD30+ (often), and a high Ki-67 proliferative index and p53 expression. Epstein-Barr virus is negative[33]. In some cases, tumor cells may show pronounced pleomorphism reminiscent of anaplastic large cell lymphoma or Hodgkin lymphoma[23]. The IELs in the non-neoplastic mucosa have the same immunophenotype as in RCeD-2. Type 2 EATL (EATL-2) is rare, and is generally not associated with a previous diagnosis of CeD[3]. While the features of non-tumoral mucosa resemble those of CeD, the tumor cells in EATL-2 have a CD3+, CD8+, CD56+ or CD4- pattern. NKp46, indicating progression from RCeD-2, has also been reported in EATL[23].

CeD AND MALIGNANCIES

The increased risk of malignant lymphomas in CeD is correlated to small bowel histopathology, and so no increased risk of lymphoma is expected in CeD patients with improved intestinal mucosal changes and with a gluten-free diet, or in potential CeD patients with an already normal intestinal mucosa[34]. Goerres *et al*[35] found intestinal UDP-glucuronosyltransferases, which are involved in the detoxification of ingested toxins and carcinogens, to be decreased in CeD, and suggested that this could

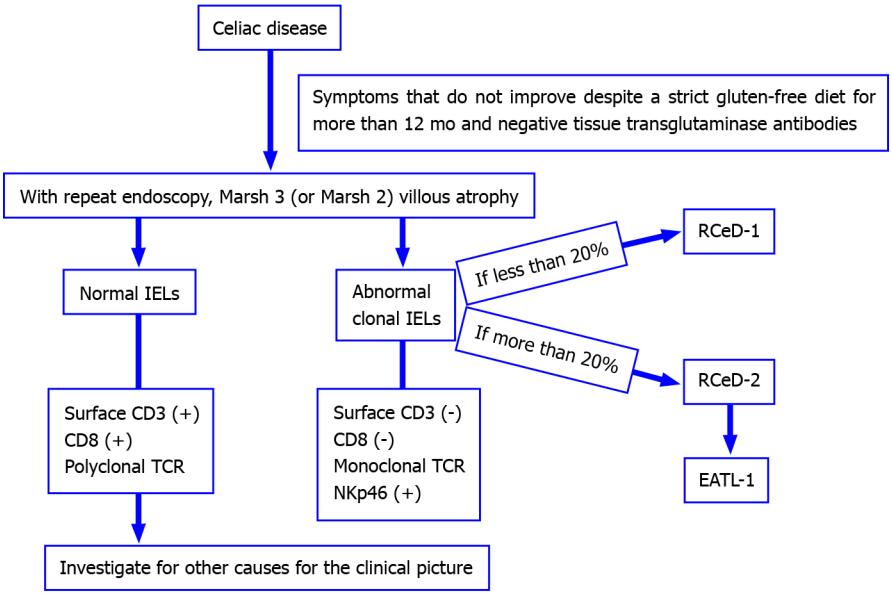

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Figure 1 Properties of refractory celiac disease type 1, type 2 and enteropathy-associated T-cell lymphoma. RCeD: Refractory celiac disease; EATL: Enteropathy-associated T-cell lymphoma; IEL: Intraepithelial lymphocytes; TCR: T cell receptor.

potentially pose a risk of cancer. Kamycheva *et al*[36] reported the leukocyte telomere length to be shorter in CeD seropositive patients, which may indicate genomic instability – a well-known predisposing factor of genetic changes and eventual carcinogenesis.

Ferguson *et al*[37] reported a 1.9 times greater risk of mortality in 653 CeD patients after a mean follow-up of 13.5 years, with the most common causes of death being lymphoproliferative disease and esophageal cancer. Freeman[38] identified 8.4% lymphoma, 1.4% small bowel carcinoma and 0.5% hypopharyngeal carcinoma in 214 patients with CeD, and reported the risk of lymphoma and small bowel adenocarcinoma to be increased especially in patients diagnosed with CeD after the age of 60 years, suggesting that risk increases the longer the diagnosis of CeD is delayed. Howdle *et al*[32] reported 13% of adenocarcinoma cases and 39% of lymphomas to have CeD.

Grainge *et al*[39] reported in their cohort study that the risk of any malignancy in CeD patients was 40% greater than in the general population, with an average follow-up of 25 years. They reported the highest risk in those with non-Hodgkin's lymphomas, with an overall incidence of 1.3 per 1000 person-years, but that the overall malignancy risk did not increase significantly 15 years after the diagnosis of CeD. Eigner *et al*[40] identified RCeD in 2.6% of 1,138 CeD patients, and reported that in 29 RCeD patients followed for 25 years, RCeD-1 developed in 1.3%, RCeD- 0.6%, EATL in 0.6% and small intestine adenocarcinoma in 0.4%, with a mortality rate of 48%. They noted further that in the preceding five years, there had been no patients diagnosed with RCeD-2, EATL or small bowel adenocarcinoma, which could be related to the increased awareness of CeD and strict adherence to a gluten-free diet.

Green *et al*[41] reported detecting small bowel adenocarcinoma in two (0.2%) and non-Hodgkin's lymphoma in five (0.4%) of 1,612 CeD patients, with EATL being found in three patients (relative risk was 300). In a meta-analysis Han *et al*[42] reported a pooled odds ratio (OR) for the risk of all malignancies of 1.25, and 1.60 for GI malignancy in CeD patients. Of the GI malignancies, esophageal cancer (pooled OR= 3.72) and small intestinal carcinoma (pooled OR = 14.41) were associated with a greater risk. Ilus *et al*[43] reported that the standardized incidence ratio (SIR) did not increase for the series as a whole in 32,439 CeD patients, but reported a decrease in breast and lung cancers, and an increase in NHL (SIR: 1.94) and small bowel cancers (SIR: 4.29) 5 years after the CeD diagnosis. In a recent study, Koskinen *et al*[44] reported that although the overall mortality in adult CeD diagnosed in 2005–2014 had not increased, mortality associated with lymphoproliferative diseases had increased, but to a lesser degree than previously reported.

Table 1 provides details of studies of malignancies in CeD patients, including those identifying and not identifying an increased risk. The malignancies associated with CeD in the case reports are presented in Table 2.

CONCLUSION

A causal relationship between CeD and EATL2 has been proven. Although its relationship with other cancer types is controversial, considering the pathogenesis of CeD, such a possibility can be considered.

Table 1 Malignancies with increased risk, or not reported in studies of patients with celiac disease

Ref.	Study design	Increased risk	No increased risk
Eigner *et al*[40]	Retrospective cohort	EATL	-
		Small bowel adenocarcinoma	
Freeman[38]	Retrospective cohort	Lymphoma	-
		Small bowel carcinoma	
		Hypopharyngeal carcinoma	
Grainge *et al*[39]	Cohort	All malignancies	-
		Non-Hodgkin's lymphoma	
Howdle *et al*[32]	Survey	Small bowel adenocarcinoma	-
		Small bowel lymphoma	
van Gils *et al*[47]	Case-control	T-cell lymphoma, predominantly EATL	Other types of lymphomas
		Small bowel adenocarcinoma	GI carcinomas
		Esophageal squamous cell carcinoma	
Anderson *et al* [48]	Retrospective cohort	Non-Hodgkin's lymphoma (but not statistically significant)	-
Green *et al*[41]	National survey	Small bowel adenocarcinoma	-
		Non-Hodgkin's lymphoma	
Han *et al*[42]	Meta-analysis	All malignancies	Other GI cancers
		Small intestinal cancers	
		Esophageal cancer	
Ilus *et al*[43]	Retrospective cohort	Non-Hodgkin lymphoma	Decreased risk of lung, pancreatic, bladder, renal and breast cancer
		Small intestinal cancer	
		Colon cancer	
		Basal cell carcinoma of the skin	
Kent *et al*[49]	Cohort	Papillary thyroid cancer	-
Lebwohl *et al* [50]	Population-based setting	-	Cutaneous malignant melanoma
Volta *et al*[51]	Cohort	-	Colon carcinoma

EATL: Enteropathy-associated T-cell lymphoma; GI: Gastrointestinal; OR: Odds ratio; RR: Relative risk.

Studies have suggested that this risk is gradually decreasing[38,39] due to the increased awareness of CeD over the years, and the widespread use of diagnostic tests and endoscopy, which have made diagnosis easier and more common. Furthermore, the increase in the availability of commercial gluten-free products has facilitated stricter compliance with gluten-free diets. Today, the follow-up of CeD patients at certain periods is recommended in CeD guidelines[1,45]. In the event of suspected non-compliance with a gluten-free diet, or when presented with symptoms, the patient is re-evaluated with CeD-specific antibodies and the presence of RCeD is investigated. The major limitation of most of the above-mentioned studies is the lack of reporting on the compliance of CeD patients with the diet "assessed from year to year" based on CeD-specific tests. Indeed, in some of the studies, the CeD diagnosis was made either together or recently in some of the patients diagnosed with lymphoma at elderly ages. For this reason, objective evaluations (monitoring with CeD-specific antibodies or measurement of gluten immunogenic peptides in urine and feces[46]) of CeD patients diagnosed in childhood will yield better results. In addition to the above, since intestinal villous atrophy improves with a gluten-free diet, an early diagnosis of CeD and a lifelong gluten-free diet are very important in preventing the formation of intestinal lymphoma and adenocarcinoma. Regular follow-ups can support patients in their compliance with a gluten-free diet.

Table 2 Malignancies associated with celiac disease in case reports

Ref.	Diagnosis of malignancies (age in years)
Ahluwalia *et al*[52]	Burkitt-like lymphoma of colon (75)
Buess *et al*[53]	EATL causing obstructive jaundice (54)
Cankurtaran *et al*[54]	Plasma cell dyscrasia (65)
Cereda *et al*[55]	1st patient: Burkitt lymphoma of the small bowel (5)
	2nd patient: Ependymoma (4)
	3rd patient: Ewing sarcoma (6)
Zunguo *et al*[56]	Large B-cell lymphoma and enteropathy-type T-cell lymphoma (65)
Fallah *et al*[57]	Adenocarcinoma of the small intestine (89)
Jafroodi *et al*[58]	Hodgkin's lymphoma (11)
Naderi *et al*[59]	Two patients: germ cell tumor (3.5 and 5)
	3rd patient: Wilm's tumor (6)
	4th patient: Acute lymphobolastic lymphoma (4.5)
	5th patient: Astrocytoma (8)
Sahin *et al*[60]	Intestinal adenocarcinoma (58)
Zullo *et al*[61]	Intestinal adenocarcinoma (77)

FOOTNOTES

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MINIREVIEWS

Breast cancer in India: Present scenario and the challenges ahead

Ravi Mehrotra, Kavita Yadav

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Abstract

Breast cancer is the commonest malignancy among women globally. From being fourth in the list of most common cancers in India during the 1990s, it has now become the first. In this review, we examine the available literature to understand the factors that contributed to the high burden of breast cancer in the country. We also provide the landscape of changes in the field of early diagnosis and the treatment modalities as well as the limitations of the Indian healthcare delivery systems (*e.g.*, delayed diagnosis, human resources and funding for treatment). This review also sheds light on the newer interventions and the future of breast cancer management keeping in mind the coronavirus disease 2019 imposed limitations.

Key Words: Breast cancer; Challenges; Prospects; India; Treatment

Core Tip: This review highlights the progress that has been made in the field of breast cancer management in India over the past few decades, in terms of addressing the various challenges of breast cancer control including diagnostic methods and treatment options. It also highlights the future of breast cancer control strategies with a focus on the coronavirus disease 2019 situation.

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INTRODUCTION

Introduction and epidemiology

Breast cancer (BC) is the commonest malignancy among women globally. It has now surpassed lung cancer as the leading cause of global cancer incidence in 2020, with an estimated 2.3 million new cases, representing 11.7% of all cancer cases[1]. Epidemiological studies have shown that the global burden of BC is expected to cross almost 2 million by the year 2030[2]. In India, the incidence has increased significantly, almost by 50%, between 1965 and 1985[3]. The estimated number of incident cases in India in 2016 was 118000 (95% uncertainty interval, 107000 to 130000), 98.1% of which were females, and the prevalent cases were 526000 (474000 to 574000). Over the last 26 years, the age-standardised incidence rate of BC in females increased by 39.1% (95% uncertainty interval, 5.1 to 85.5) from 1990 to 2016, with the increase observed in every state of the country[4]. As per the Globocan data 2020, in India, BC accounted for 13.5% (178361) of all cancer cases and 10.6% (90408) of all deaths (Figure 1 and Figure 2) with a cumulative risk of 2.81[5].

Current trends point out that a higher proportion of the disease is occurring at a younger age in Indian women, as compared to the West. The National Cancer Registry Program analysed data from cancer registries for the period from 1988 to 2013 for changes in the incidence of cancer. All population-based cancer registries have shown a significant increase in the trend of BC[6]. In India in 1990, the cervix was the leading site of cancer followed by BC in the registries of Bangalore (23.0% *vs* 15.9%), Bhopal (23.2% *vs* 21.4%), Chennai (28.9% *vs* 17.7%) and Delhi (21.6% *vs* 20.3%), while in Mumbai, the breast was the leading site of cancer (24.1% *vs* 16.0%). By the years 2000-2003, the scenario had changed, and breast had overtaken as the leading site of cancer in all the registries except in the rural registry of Barshi (16.9% *vs* 36.8%). In the case of BC, a significant increasing trend was observed in Bhopal, Chennai and Delhi registries[7].

When it comes to the 5-year overall survival, a study reported it to be 95% for stage I patients, 92% for stage II, 70% for stage III and only 21% for stage IV patients[8]. The survival rate of patients with breast cancer is poor in India as compared to Western countries due to earlier age at onset, late stage of disease at presentation, delayed initiation of definitive management and inadequate/fragmented treatment[9]. According to the World Cancer Report 2020, the most efficient intervention for BC control is early detection and rapid treatment[10]. A 2018 systematic review of 20 studies reported that BC treatment costs increased with a higher stage of cancer at diagnosis. Consequently, earlier diagnosis of BC can lower treatment costs[11].

EARLY DETECTION AND SCREENING PROGRAMMES

Success and failure of screening programs depend on several factors ranging from the presence of proper guidance manuals, development and usage of an appropriate instrument for diagnosis to proper implementation and availability of adequate human resources. Another factor is the efficacy of the screening test in avoiding the risk of false positives and unnecessary biopsies and surgeries[12]. Organised screening programmes provide screening to an identifiable target population and use multidisciplinary delivery teams, coordinated clinical oversight committees and regular review by a multi-speciality evaluation board to maximise the benefit to the target population[13]. Screening strategies are moving towards a risk-based approach rather than a broad age-based and sex-based recommendation. To use this risk-based approach, India needs to assess risk factors and incorporate this information into BC screening[14].

A recent study from Mumbai has reported that clinical breast examination conducted every 2 years by primary health workers significantly downstaged breast cancer at diagnosis and led to a nonsignificant 15% reduction in breast cancer mortality overall (but a significant reduction of nearly 30% in mortality in women aged ≥ 50)[15]. Mammography sensitivity has been reported to vary from 64% to 90% and specificity from 82% to 93%[16]. Indian women have more dense breasts, and there is a lack of adequate mammography machines and trained manpower. This may result in false positives and over-diagnosis. Digital mammography uses computer-aided detection software but remains costly. It is due to these reasons that mass-scale routine mammography screening is not a favoured option for a transitioning country like India.

Ultrasonography has an overall sensitivity of 53% to 67% and specificity of 89% to 99%[17,18] and might be particularly helpful in younger women (aged 40 to 49 years). However, the requirement of trained professionals to perform and interpret ultrasound is a major hurdle. Though breast self-examination is not accepted as an early detection method for BC, this technique, if used diligently and skilfully, can serve as a useful adjunct to making the woman aware of her normal breast[19].

Understanding India-specific differences by utilising genomics may enable the identification of women at high risk of developing cancer, where targeted screening may be cost-effective. There is an urgent need to identify Indian-specific genetic/epigenetic biomarkers. These may have the potential to be used as biomarkers for early detection at the screening stage[20].

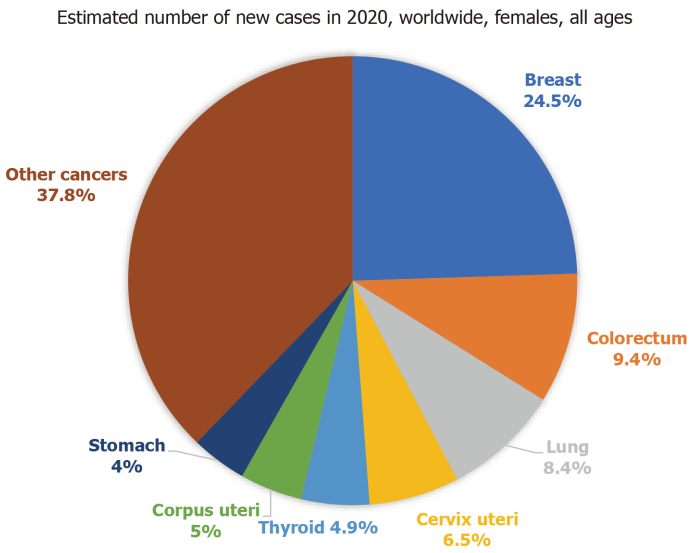

Figure 1 World Health Organization: Estimated number of new cancer cases in 2020, worldwide. Image available under Common Creative License [68] (Available at https://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf).

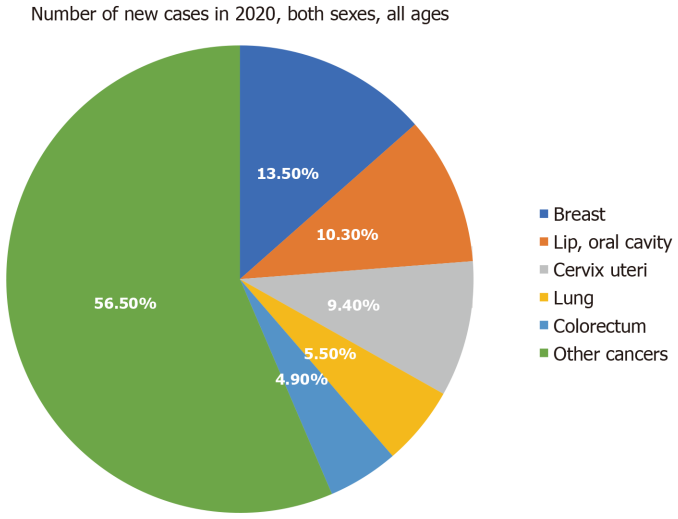

Figure 2 World Health Organization Globocan 2020 India. Image available under Common Creative License[68] (Available at https://gco.iarc.fr/today/data/factsheets/cancers/20-Breast-fact-sheet.pdf).

TREATMENT OPTIONS IN PAST AND PRESENT

Management of BC is multidisciplinary and has come a long way. In the past, the widely used treatment option was mastectomy followed by adjuvant chemotherapy for locally advanced BC, triple-negative breast cancer and HER2neu expressing tumours (human epidermal growth factor receptor 2). At present, it includes a loco-regional approach (targeting only the tumour with the help of surgery and radiation therapy) and a systemic therapy approach that targets the entire body. The systemic therapy includes endocrine therapy for hormone receptor-positive disease, chemotherapy, anti-HER2 therapy for HER2 positive disease, bone stabilising agents, polymerase inhibitors for BRCA (breast cancer gene) mutation carriers and, recently, immunotherapy. However, the majority of patients still undergo primary ablative surgical procedures. Gene expression profiling in hormone receptor-positive disease is also a promising option but has financial implications.

From using drugs like cyclophosphamide, methotrexate, *etc.* in the 1970s for chemotherapy to using their modifications like anthracycline-based combination chemotherapy protocols in the 1980s and 1990s, we have come a long way. Taxanes are the newer additions that show a promising future. The radiation treatment of BC has evolved from 2D to 3D conformal radiotherapy and accelerated partial breast irradiation, aiming to reduce normal tissue toxicity and overall treatment time[21]. The newer additions, viz. intensity-modulated radiation therapy and deep inspiration breath-hold, are still inaccessible to many. The same is the case with brachytherapy.

The outcomes with triple-negative breast cancer are poor, and the treatment options are mainly restricted to systemic chemotherapy. Immunotherapy, poly adenosine diphosphate-ribose polymerase inhibitors (poly(adenosine diphosphate-ribose) polymerase) and antibody-drug conjugates have the potential to change the current scenario of BC treatment. One important point that should be considered while planning the treatment is that there is a lot of hype regarding the newer drugs that flood the market, however with little or no difference in the survival benefit. Hence it is important to choose wisely.

The field of breast surgery has also evolved from total mastectomy to breast conservation therapy to oncoplastic breast surgery. The rapidly advancing field of oncoplastic breast surgery offers a pragmatic alternative to total mastectomy and breast conservation therapy. It is currently nascent but expected to attain mainstream status in the near future as oncoplastic breast surgery has economic feasibility and cost-effectiveness and is well suited for a low-resource setting such as India[22].

CHALLENGES IN BC CONTROL

Delay in seeking healthcare

Continuously increasing BC prevalence is just the tip of the iceberg. There are many underlying problems that contribute to this mounting burden. In India, nearly 60% of BC cases are diagnosed at stage III or IV of the disease[23]. Most of the patients present to the healthcare facility only when there is a large palpable mass or secondary changes like local skin/chest wall changes are visible. Women tend to ignore the minor symptoms and do not show up at the hospital until it is unbearable, owing to their household responsibilities. Other factors that may contribute to the late presentation include a lack of awareness about the disease, especially in rural areas. This also leads to fewer women performing a self-breast examination, opting for a periodic examination by a healthcare worker or mammography for BC screening, despite it being available for free in a few government hospitals. For those willing to pay for it, mammography is available in private hospitals. This lack of awareness regarding the risk factors and early detection methods of BC is unfortunately even prevalent in 49% of healthcare workers[24].

The initial manifestation of BC, *i.e.* a lump, is generally not associated with pain, which further adds to the delay in seeking treatment in 50% to 70% of the cases in rural areas[25]. Other factors that may influence the early detection and treatment of BC are the presence of a diagnostic/treatment facility in the nearby area, patient's preference and trust in the healthcare provider, amount of time required for travelling to the service centre and amount and availability of money that can be spent on the treatment. However, this grim situation has slowly started to change due to various awareness campaigns, and now women have slowly started to understand and value their health. Another deterring factor in seeking early care is the stigma of social embarrassment and isolation. Women not only fear death and contagion by cancer but also fear that their and their family's reputation would suffer if people knew of their cancer diagnosis, including potential difficulties in their daughter's wedding. It is also a widespread assumption that cancer, especially in the private parts (breast and genitals) is linked to "bad" and "immoral behaviour"[26]. The issue of social stigma urgently needs to be addressed through awareness campaigns, as it not only jeopardises early diagnosis but also the treatment-seeking behaviour of women with symptoms of BC.

Delay from the healthcare provider's side

On average, more than 12 wk of delay is seen in diagnosis and treatment in 23% of patients[27]. A study examined provider's delay (defined as the period between the first consultation and diagnosis) and observed that the mean provider delay was 80 d in rural areas and 66 d in urban areas[28]. More than half of the women were observed to have a delay of more than 90 d in seeking care. The patient-related delay was observed to be 6.1 wk, and the system-related delay was 24.6 wk with a mean total delay of 29.4 wk in treatment. This led to a poor prognosis[29]. These delays may be attributed partly to the ever-increasing patient load on the health care system and competing priorities.

High attrition rates

After crossing all these hurdles, once the diagnosis is done and treatment is started, there are further hazards. In 1990, India's facilities for the diagnosis and treatment of cancer were far behind recommendations[30]. Even today, due to a large variation in the health care standards between regions, the quality of treatment for BC patients varies from pathetic to world-class. Few patients are treated at well-equipped centres in a protocol-based manner, while some are subjected to numerous compromises. Fortunately, BC is curable if detected early, but due to various underlying factors, improper treatment provided locally by non-oncologists without standard oncology expertise may lead to the mismanagement of BC cases. At the same time, patients with advanced, metastatic incurable cancer may require only palliative care are referred to tertiary cancer centres. This leads to the improper use of limited, valuable resources.

Another issue that adds to the high attrition rates/loss to follow-up of BC treatment is an unacceptable out-of-pocket expenditure, which is three times higher for private inpatient cancer care in India[30]. More than half the patients from low-income households spend > 20% of their annual household expenditure on BC treatment, leading to catastrophic results[31]. An analysis of three public insurance schemes for anticancer treatment in India published in 2018 revealed inconsistencies in the selection of reimbursed treatments. The reimbursed amount was usually found to be insufficient to cover the total cancer chemotherapy costs, leading to an average budget shortage of up to 43% for BC treatment[32]. Cancer insurance policies can significantly reduce the financial burden caused by out-of-pocket expenditure and prevent catastrophic health expenditure, distress financing and even bankruptcy. However, a study by Singh *et al*[33] to understand the use of health insurance in India reported a lack of awareness regarding the use of these schemes as the key reason for the low penetration of health insurance policies.

Shortage of resources/skewed distribution of available limited resources

Another problem is a shortage of manpower. India has just over 2000 oncologists for 10 million patients, and the number of oncologists is unevenly spread, being lower in semi-urban and rural areas. Although nearly 70% of the Indian population lives in rural areas, about 95% of facilities for cancer treatment exist in the urban areas of the country. There are also regional variations. About 60% of specialist facilities are in Southern and Western India, whereas more than 50% of the population lives in the Northern, Central and Eastern regions, distorting service provision[34].

There are currently 57 courses for radiotherapy technologists and about 2200 certified radiation technologists in practice in India[35]. As per a recent study, India has just 10% of the total requirement of 5000 radiation therapy units indicating a shortfall of > 4500 machines. The World Health Organization recommends at least one teletherapy unit per million population, and there is a shortfall of 700 teletherapy units[35]. If we look at the treatment infrastructure, at least half of patients with cancer will be judged to need radiotherapy at some point. Yet only 26% of the population, living in the Eastern region of India, have immediate access to only 11% of radiotherapy facilities[36]. Nearly 40% of hospitals in India are not adequately equipped with advanced cancer care equipment. Very few centres in the country provide integrated surgical and chemoradiation for BC. Nearly 75% of the patients in the public sector do not have access to timely radiotherapy[37].

All of these factors aid in raising the overall cost of BC treatment for the common person. They are forced to make out-of-pocket cancer care payments, as most of the patients have to bear the cost of therapy. The government facilities are inadequate in number to cater to a large number of patients. Thus, patients are required to go for treatment in major cities along with their attendants, resulting in loss of livelihood of both the patient and her attendants. There is an urgent need to establish a larger number of cancer care facilities accessible to those living in rural areas so that the gap of cancer detection and treatment services may be bridged.

RAYS OF HOPE/WAY FORWARD

The integrative approach

The rising human cost, both social and economic, of BC underscores the need for more holistic, multidimensional approaches that encompass the cancer care continuum including prevention, early detection, treatment, palliation and survivorship. A balanced approach is necessary to integrate traditional medical practices into mainstream oncology practice, starting with a meaningful discussion among all the relevant stakeholders and identifying the areas where the benefits of a complementary approach are beyond doubt[38]. Exercise has been proven to be an effective, safe and feasible tool in combating the adverse effects of treatment, prevents complications and decreases the risk of BC-specific mortality[39]. A recent review has reported evidence that diet-related and physical activity-related interventions for the primary prevention of BC are cost-effective[40]. Such lifestyle modifications need to be included in mainstream treatment planning.

The internet era

Screening programmes in high-income countries that have increased patient participation have done so with high-quality and periodic education programmes with campaigns tailored to the specific cultural context of a community[41]. This can only be achieved by creating and sustaining the level of awareness among the general population. One effective way of doing that is providing information on BC to the relatives/patients in the hospital setting. However, due to the ever-increasing patient burden, it is not always possible for healthcare providers to give appropriate time and counselling to the people. The distribution of pamphlets with valuable information may be the cause. In recent times, the Internet is being increasingly used as a reliable source to seek health-related information[42,43]. A lot of people search for information online. Having a credible source of information that can be revisited as and when required by the people is always a plus. Keeping these things in mind a website 'Cancer India' was developed by the National Institute of Cancer Prevention and Research providing comprehensive

information on common cancers in India, including BC, in layman's language (available in English and Hindi). A recent evaluation by this group reported that the website managed to serve the intended purpose of improving cancer awareness with reasonable success[44].

Involvement of community health workers

Community participation with the engagement of the health system and local self-government are required for implementing a comprehensive cancer screening strategy. A BC screening program using local volunteers for early detection is feasible in low-income settings, thereby improving survival[45]. Community health workers can play an important role in the early detection of BC in low- and middle-income countries, with responsibilities including awareness-raising, conducting clinical breast examinations, making referrals and supporting subsequent patient navigation. However, this promise can only be turned into genuine progress if these activities are appropriately supported and sustained. This will involve adopting contextually appropriate early detection initiatives that are embedded within the broader health system where community health workers are appropriately trained, equipped, paid and supported with appropriate links to specialist oncology services. A recent study reported the effectiveness of the Extension for Community Healthcare Outcomes model training program in reaching primary care physicians across the country and improving their knowledge and skills related to screening for breast, oral and cervical cancer[46].

Early diagnosis

With advancements in molecular diagnostics and therapeutics, newer non-invasive prognostic biomarker tests to detect BC at a very early stage, such as digital breast tomosynthesis and breast biopsy techniques, are becoming available[47]. Above all, early detection programmes in low- and middle-income countries must make provisions for every individual at risk of BC. This will mean considering the needs of the hardest individual to reach first, so that no woman is left behind in the goal to end unjust and untimely deaths attributable to the leading cause of female mortality in low- and middle-income countries[48].

Out of pocket expenditure

To address the issue of out-of-pocket expenditure and to reach out to the poorer section of the society, a scheme called Pradhan Mantri Jan Arogya Yojana under the new universal healthcare programme named "Ayushman Bharat" has been recently launched in India. It is the largest health assurance scheme in the world that aims at providing a health cover of Rs. 5 Lakhs (6814 USD) per family per year for secondary and tertiary care hospitalisation to over 10.74 crores (approximately 107 million) poor and vulnerable families (approximately 500 million beneficiaries) that form the bottom 40% of the Indian population[49]. The Indian government's efforts to bring anti–HER2 drug under-pricing regulations have enhanced access and improved outcomes. The launch of low-cost T-DM1 (the antibody-drug conjugate trastuzumab emtansine) and anti–HER2 therapy biosimilars is keenly awaited. Linking cancer registry data with Ayushman Bharat, mortality databases and the Hospital Information System could improve cancer registration, follow-up and outcome data[50].

Newer initiatives

There are several health-tech start-ups that aid in different stages of cancer care. For example, Niramai® uses machine learning and big data analytics to develop low-cost diagnostics for BC[51]. Oncostem® uses multi-marker prognostics tests that aid in personalised treatment[52], and UE Lifesciences® uses contactless and radiation-free handheld screening devices[53] that may come in handy during coronavirus disease 2019 (COVID-19) times. Panacea® and Mitra Biotech®[54] are start-up companies that deal with providing precision therapies. Tumourboard® and Navya Network®[55] provides affordable and precise consultations. There are several drug-patient assistance programs from pharmaceutical companies like Roche®, Novartis®, Dr Reddys®, *etc.* that help in following patient-centric care from beginning to end. The BC Initiative 2.5 is a global campaign to reduce disparities in BC outcomes and improve access to breast health care worldwide. It is a self-assessment toolkit that can help countries conduct a comprehensive breast health care situational analysis[56] Apart from these, patient navigation strategies are also rapidly growing and evolving concepts. 'Kevat' is the first initiative in India to create a trained task force to facilitate the cancer patient's journey from entry to the hospital to follow-up. It is a nascent area of speciality in cancer care that is set to target the pressing need of well-trained patient navigators in onco-care[57].

Apart from these, the World Health Organization, on March 9, 2021, introduced a Global Breast Cancer Initiative to reduce global breast mortality by 2.5% by 2040. The aim is to reduce 2.5 million global deaths, particularly in low-income countries, where the progress to tackle the disease has been relatively slow. An evidence-based technical package will be provided to countries as part of the initiative[58]. Such initiatives instil faith in the future for better breast cancer management.

COVID-19 challenge

The COVID-19 pandemic has challenged the prioritisation of various diseases in healthcare systems

across the globe, and BC patients are no exception. It impacted their access to physicians, medication and surgeries. Nearly 70% of patients could not access life-saving surgeries and treatment. Chemotherapy treatments and follow-ups were postponed due to lockdowns[59,60]. A recent study reported that the average monthly expenditure of cancer patients had increased by 32% during the COVID-19 period while the mean monthly household income was reduced by a quarter. More than two-thirds of the patients had no income during the lockdown, and more than half of the patients met their expenditure by borrowing money. The incremental expenditure coupled with reduced or no income due to the closure of economic activities in the country imposed severe financial stress on patients with BC[61].

It became of utmost importance to balance between the benefits and risks associated with BC treatment[62]. There are different treatment modalities and each case is different. The shorter duration of radiotherapy, transient spacing of chemotherapy in metastatic BC setting, if deemed feasible, oral hormone therapy to delay surgery and judicious use of immunomodulators are some recent guidelines in evidence-based practice in this unprecedented crisis[63]. The core idea is to delay the surgery until the pandemic is over whenever feasible. Additionally, apart from healthcare management, one area that has suffered tremendously due to the infectious outbreak is cancer research[64]. The estimated decrease in cancer funding in India ranges from 5% to 100%, as many funding agencies have cancelled calls for funding. The private/charity sector is the worst hit, with an estimated decrease of more than 60% of its funding[65]. A robust health system is a prerequisite for providing the facilities for the treatment of BC that is diagnosed through the early detection programmes, whether through screening or the presence of symptoms[66]. Of course, it goes without saying that a proper evaluation of the programmes will not only allow improvement of quality of services but also generate valuable evidence on the effectiveness of screening and early diagnosis in the countries "in transition"[67].

CONCLUSION

To conclude, the BC burden is rising at a rate much faster than it was a decade ago. Acknowledging that BC is one of the foremost cancers in India now would be the first step towards making people cognizant of the disease. It is fast developing into a public health crisis, and society's discomfort to talk about women's bodies has made the situation even worse. To combat the consequences as a country, better preparedness is essential. A robust awareness campaign and effective implementation of a national cancer screening program are the need of the hour. We also need to stand up and deliver on the healthcare front. The shortage of skilled manpower and infrastructural requirements need to be met, and for this, the total healthcare budget of the country needs to be increased. In the jargon of the challenges of BC control, prioritising the adoption of a preventive approach and early detection would go a long way. Another important aspect is the country's preparedness for unprecedented events like COVID-19, for which there should be a separate provision to deal with public health disasters. Creating a cadre of trained medical and paramedical professionals, efficient utilisation and timely upgrading of skills of the existing healthcare workforce along with adopting newer technologies would further the cause of BC control.

FOOTNOTES

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MINIREVIEWS

Immunotherapy in triple-negative breast cancer: A literature review and new advances

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Abstract

Triple-negative breast cancer (TNBC) is a highly complex, heterogeneous disease and historically has limited treatment options. It has a high probability of disease recurrence and rapid disease progression despite adequate systemic treatment. Immunotherapy has emerged as an important alternative in the management of this malignancy, showing an impact on progression-free survival and overall survival in selected populations. In this review we focused on immunotherapy and its current relevance in the management of TNBC, including various scenarios (metastatic and early -neoadjuvant, adjuvant-), new advances in this subtype and the research of potential predictive biomarkers of response to treatment.

Key Words: Triple-negative breast cancer; Early disease; Immunotherapy; Biomarkers; Metastatic disease

Core Tip: Triple-negative breast cancer (TNBC) is an exceptionally heterogeneous disease and historically a cancer with limited treatment options other than chemotherapy. Recent advances in immunotherapy has changed the standard of care in selected groups, especially in metastatic TNBC. This article review continues the detailed, updated and comprehensive literature review regarding immunotherapy in TNBC, including the discussion of clinical trials in different scenarios (metastatic, neoadjuvant, adjuvant) and potential biomarkers to provide useful knowledge for medical oncologists and the medical community. Our goal is sharing updated information for TNBC which is considered an overlooked population with an enormous necessity of novel treatments and biomarkers.

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INTRODUCTION

Triple-negative breast cancer (TNBC) which effects approximately 15 - 20% of all patients, is a heterogeneous, complex disease with a more aggressive behavior than other subtypes of breast cancer. It is associated with a high incidence of visceral metastasis (predominance of hepatic, pulmonary and central nervous system metastasis), a high risk of early recurrence and a worse prognosis[1]. Unlike other subtypes, historically, TNBC has had no other systemic treatment options other than chemotherapy which has been the cornerstone of treatment for many years. However, this has recently changed with the introduction of immunotherapy in patients with programmed death ligand 1 (PD-L1) expressing tumors, both in unresectable locally advanced/metastatic disease. In the neoadjuvant setting, the use of immunotherapy has recently been approved[1].

Based on efforts in genetic studies, breast cancer was divided into molecular subtypes. Perou *et al*[2] proposed a classification based on expression patterns, subdivided into 4 clinical molecular subtypes (luminal A, luminal B, HER2 enriched and basal-like). Most basal-like tumors are included in TNBCs (they represent 70%-80% of the TNBCs)[3]. Lehmann *et al*[4] identified 6 different subtypes using DNA and RNA profiles in TNBC ["basal-like 1" (BL1), "basal-like 2" (BL2), "immunomodulatory" (IM), "mesenchymal" (M), "mesenchymal stem-like" (MSL) and "luminal androgen receptor" (LAR)] each with particular characteristics. BL1 and IM tumors have a higher sensitivity to DNA-damaging agents such as platinum and are associated with a young age at diagnosis. They are also the subtype with the highest pathological complete response (pCR) rate (65.6%) followed by BL2 (36.4%) in a cohort of patients treated with platinum-based neoadjuvant therapy (n = 97). The LAR subtype has the lowest pCR rate (21.4%)[4].

Although breast cancer has traditionally been considered a non-immunogenic tumor, multiple studies have shown that TNBC can stimulate the immune system. Compared with luminal breast cancer, TNBC has a higher tumor mutational burden (TMB), elevated levels of PD-L1 expression and increased levels of tumor infiltrating lymphocytes (TILs) in the tumor microenvironment which are associated with higher rates of pCR to neoadjuvant chemotherapy and efficacy to immunotherapy which justifies the use of immunotherapy in this subtype[5].

Due to advances in the molecular characterization of TNBC, with addition of immunotherapy, new therapeutic agents including poly ADP-ribose polymerase-1 (PARP) inhibitors, tyrosine kinase inhibitors (TKI), checkpoint inhibitors, antiandrogens, antibody-drug conjugates (ADC) and other targeted therapies are being researched. Moreover, ongoing trials are evaluating immunotherapy (immune checkpoint inhibitors) in combination with PARP inhibitors in a series of cancers including BC [6].

IMMUNOTHERAPY AGENTS APPROVED IN TNBC

The high mutational burden of the TNBC was determined to lead to the synthesis of abnormal proteins, acting as "neoantigens" which will be recognized by the antigen presenting cells and would initiate an antitumor immune response[7].

Early-stage TNBC has a high TIL infiltrate but breast cancer has not traditionally been considered immunogenic. Recent trials demonstrate TIL infiltrate has a high expression of PD-1 (and other inhibitory checkpoint molecules). TNBC has potential therapeutic targets such as immune checkpoint inhibitors (ICIs) (anti-PD-1/PD-L1 agents) in metastatic and the early-stage scenario[8] (Table 1).

Table 1 Randomized phase II/III immunotherapy trials en triple-negative breast cancer

Scenario	Trial	Phase	*n*	Intervention	Recruitment Status	Magnitude of clinical benefit		
						PFS (mo)	OS (mo)	Additional information
Neoadjuvant	NCT03639948	II	100	Carboplatin + Docetaxel + Pembrolizumab	Recruiting			
	NCT03289819	II	50	Pembrolizumab + Nab-paclitaxel → Pembrolizumab + Epirubicin and Cyclophosphamide	Recruiting			
	NCT03356860 (B-IMMUNE)	II	57	Paclitaxel + Epirubicin + Cyclophosphamide + Durvalumab	Recruiting			
	NCT02685059 (GeparNuevo) (June 2018)	II	174	Epirubicin + Nab-paclitaxel + Cyclophosphamide + Durvalumab	Active, no recruiting	-	-	
				Population: Early TNBC				pCR was increased to 53.4% with Durvalumab *vs* 44.2% with chemotherapy alone, not being statistically significant ($P = 0.048$) In the PD-L1 (+) subgroup: pCR 58% *vs* 50.7% ($P = 0.363$) pCR was increased in patients with high levels of TILs y TMB-H ($P < 0.01$) 3-yr iDFS was 84.9% with durvalumab *vs* 76.9% with placebo (HR: 0.54, 0.27-1.09, $P = 0.0559$); 3-yr DDFS 91.4% *vs* 79.5% (HR: 0.37, 0.15-0.87, $P = 0.0148$); 3-yr OS: 95.1% *vs* 83.1% (HR: 0.26, 0.09-0.79, $P = 0.0076$)
Neoadjuvant/Adjuvant	NCT03036488 (KEYNOTE-522) (August 2020)	III	1174	Carboplatin + Paclitaxel + AC (anthracycline + cyclophosphamide) +/- Pembrolizumab → Adjuvant Pembrolizumab	Active, no recruiting	-	-	Co-primary endpoints were pCR and EFS
				Population: Early TNBC				pCR: 64.8% in Pembro group *vs* 51.2% with placebo ($P < 0.001$) The benefit of Pembro in pCR was consistent in all subgroups, including PD-L1 (+): pCR 68.9% *vs* 54.9% ($P < 0.001$) A statistically benefit was observed in EFS (HR: 0.63, 0.48-0.82) Pembro showed a favorable trend in OS (HR: 0.72, 0.52-1.02)
	NCT02620280 (NeoTRIPaPDL1)	III	280	Carboplatin/nab-paclitaxel +/- Atezolizumab → anthracycline (AC/EC)	Active, no recruiting	-	-	Primary endpoint was pCR

	(December 2019)			Population: Early TNBC		The pCR rates were not statistically significant between both groups: 43.5% with atezolizumab *vs* 40.8% with chemotherapy alone A multivariate analysis showed that the only variable associated with pCR was the PD-L1 (+) status: pCR 51.9% *vs* 48% ($P < 0.0001$) These results differ from KEYNOTE-522, where pembrolizumab achieved significant rates of pCR in a similar population
	NCT03281954	III	1520	Doxorubicin + Cyclophosphamide + Paclitaxel + Carboplatin +/- Atezolizumab → Atezolizumab	Recruiting	
	NCT03197935 (IMpassion031) (September 2020)	III	204	AC (doxorubicin + cyclophosphamide) + Nab-paclitaxel +/- Atezolizumab → Adjuvant Atezolizumab Population: Early TNBC	Active, no recruiting	pCR was 58% in Atezolizumab group *vs* 41% in placebo group ($P = 0.0044$) In the PD-L1 (+) population, pCR was 68.8% in the Atezolizumab group *vs* 49.3% in the placebo group ($P = 0.021$) A favorable trend was obtained in EFS (immature data) (HR: 0.76, 0.40 -1.44) In patients with early TNBC, neoadjuvant treatment of Atezolizumab + nab-paclitaxel and an anthracycline-based regimen achieve higher rates of pCR, with an acceptable safety profile
Adjuvant (for patients with residual disease after neoadjuvant chemotherapy)	NCT02954874	III	1000	Pembrolizumab *vs* observation	Recruiting	
	NCT03756298	II	284	Capecitabine +/- Atezolizumab	Recruiting	
Adjuvant	NCT03498716 (IMpassion030)	III	2300	Paclitaxel → dd Doxorubicin/Epirubicin + Cyclophosphamide +/- Atezolizumab	Recruiting	Primary endpoint was iDFS Secondary endpoints were iDFS according to PD-L1 status and nodal affectation, OS, safety, y health related to a QoL
	NCT02926196 (A-Brave)	III	335	Avelumab *vs* observation	Recruiting	This trial evaluates patients in two groups: (1) Primary TNBC patients who completed surgery followed by adjuvant therapy; and (2) Primary TNBC patients with residual disease after neoadjuvant chemotherapy (did not achieve pCR) The first and second co-primary endpoints are DFS in all patients and DFS in B group
Locally advanced or mTNBC	NCT02768701	II	40	Cyclophosphamide + Pembrolizumab	Active, no recruiting	

	NCT03121352	II	30	Carboplatin, Nab-paclitaxel y Pembrolizumab	Recruiting			
	NCT02499367 (TONIC)	II	67	Control or irradiation 3 x 8 Gy or oral cyclophosphamide or Cisplatin or Doxorubicin → anti-PD-1 (Nivolumab)	Active, no recruiting			Five cohorts were included in the randomization, all followed by nivolumab Overall, the ORR was 20% Most responses were observed with cisplatin (ORR: 23%) and doxorubicin (ORR: 35%)
	NCT02819518 (KEYNOTE-355) (December 2020)	III	858	Nab-paclitaxel or Paclitaxel or Carboplatin/Gemcitabine +/- Pembrolizumab Population: First-line mTNBC	Active, no recruiting	9.7 *vs* 5.6 (HR: 0.82) in CPS ≥ 10	-	Co-primary endpoints were PFS and OS (this latter is pending outcome) Pembro treatment was statistically significant only for patients with high levels of PD-L1 (expressed in CPS ≥ 10) Pembro + chemotherapy showed a significant increase in PFS among mTNBC patients A recent update showed that KEYNOTE-355 trial met primary endpoint of OS in patients with mTNBC whose tumors expressed PD-L1 (CPS ≥ 10)
	NCT02555657 (KEYNOTE-119) (September 2019)	III	600	Capecitabine, Eribulin, Gemcitabine, or Vinorelbine *vs* Pembrolizumab Population: Second and third-line mTNBC	Active, no recruiting	2.1 *vs* 2.1 (HR: 1.14)	12.7 *vs* 10.7 (HR: 0.78)	Pembro did not show improvement in OS or PFS as 2L/3L of treatment for mTNBC *vs* chemotherapy (OS: 9.9 mo *vs* 10.8 mo, HR: 0.97, 0.82- 1.15) OS in tumors with CPS > 10: 12.7 mo *vs* 11.6 mo (HR: 0.78, 0.57-1.06) A greater benefit was obtained in OS/PFS in tumors with high levels of PD-L1 (expressed in the CPS score) Pembro was well tolerated and had less adverse events compared with chemotherapy
	NCT02447003 (KEYNOTE-086) (March 2019)	II	285	Pembrolizumab monotherapy	Active, no recruiting	-	-	Primary endpoint: ORR in the total population and PD-L1 (+) ORR was 5.3% in the total population, and 5.7% in the PD-L1 (+) population Pembro demonstrated antitumor activity in patients previously treated with mTNBC (≥ 1 systemic treatments)
	NCT02425891 (IMpassion130) (November 2018)	III	902	Atezolizumab + Nab-paclitaxel (comparator: placebo + Nab-paclitaxel)	Active, no recruiting	7.5 *vs* 5.5 (HR: 0.62, $P < 0.001$)	25.0 *vs* 15.5 (HR: 0.62)	In the analysis of the ITT population, the median PFS was 7.2 mo *vs* 5.5 mo (HR: 0.80, $P = 0.002$). In PD-L1 (+) patients, the median PFS was 7.5 mo *vs* 5.5 mo (HR: 0.62, $P < 0.001$)

				Population: First-line mTNBC				In the analysis of the ITT population, the median OS was 21.3 mo *vs* 17.6 mo (HR: 0.84, P = 0.08). In PD-L1 (+) patients, the median OS was 25.0 mo *vs* 15.5 mo (HR: 0.62) Final analysis showed that OS benefit with atezolizumab + nab-paclitaxel in the ITT population was not statistically significant, but a clinically meaningful OS benefit was observed in PD-L1 IC-(+) patients
	NCT03125902 (IMpassion131) (September 2020)	III	600	Paclitaxel +/- Atezolizumab (comparator: placebo + paclitaxel) Population: First-line mTNBC	Active, no recruiting	5.7 *vs* 6.0 (HR: 0.82, P = .20) in PD-L1 (+) population	22.1 *vs* 28.3 (HR: 1.12) in PD-L1 (+) population	Primary endpoint was PFS In the ITT population, the median PFS was 5.7 mo in atezolizumab group *vs* 5.6 mo in placebo group (HR: 0.86) OS: 19.2 mo *vs* 22.8 mo (HR: 1.11, 0.87-1.42) The 2-yr OS rates were 51% and 49% in placebo and atezolizumab groups, respectively
	NCT03371017 (IMpassion132) (early recurrence)	III	350	Carboplatin + Gemcitabine or Capecitabine +/- Atezolizumab	Recruiting			Primary endpoint was OS Estimated completion date: July 2023

CPS: Combined positive score; dd: Dense dose; DDFS: Distant-disease free survival; DFS: Disease-free survival; EFS: Event-free survival; HR: Hazard ratio; IC: immune cells; iDFS: Invasive disease-free survival; ITT: Intention to treat; mTNBC: Metastatic triple-negative breast cancer; pCR: Pathological complete response; PFS: Progression-free survival; ORR: Objective response rate; OS: Overall survival; QoL: Quality of life; TNBC: Triple-negative breast cancer.

In the early stage scenario there are considerations for the addition of immunotherapy to chemotherapy in the neoadjuvant setting: the benefit of improving the pCR rates (KEYNOTE-522, IMpassio031), and the risks regarding toxicities (immune related adverse events in a potentially curable setting) and costs.

Atezolizumab

Atezolizumab is a humanized anti-PD-L1 monoclonal antibody, non-glycosylated IgG1 that binds to PD-L1 and blocks interaction with PD-1 and B7.1 (a co-stimulatory protein on the cell surface) that induces a reactivation of the antitumor immune response without antibody-induced cellular cytotoxicity [9].

Atezolizumab monotherapy in mTNBC: A phase I study (Schmid *et al*[10], 2017) that evaluated the safety and tolerability of atezolizumab single-drug (primary endpoints), demonstrated an antitumor activity and safety with the use of atezolizumab in patients with mTNBC (n = 116). It was also observed that the greatest benefit was in patients who received atezolizumab in the first line and among those with high levels of TILs and PD-L1 immune cells (IC)[10].

Other measured endpoints were overall survival (OS) (41% at 1 year, 19% at 2 years, and 16% at 3 years) and the PD-L1 IC ≥ 1% was associated with a higher objective response rate (ORR) (12% *vs* 0%) and higher OS (10.1 mo *vs* 6 mo, respectively). Atezolizumab was well tolerated and provides clinical

benefit in patients with mTNBC. 100% of the patients who responded to atezolizumab were alive at 1 year *vs* 38% of non-responders[10].

Atezolizumab + nab-paclitaxel in mTNBC (IMpassion130): The IMpassion130 (November 2018), phase III, randomized trial evaluated patients with mTNBC or unresectable locally advanced disease without previous treatment (n = 902) and regardless of PD-L1 expression, who were randomized (in a 1:1 ratio) to receive nab-paclitaxel (100 mg/m^2 on days 1, 8 and 15 every 28 d) in association with atezolizumab (840 mg IV on days 1 and 15 every 28 d) or with placebo until disease progression or limiting toxicity [11]. The two primary end points were progression-free survival (PFS) [in the intention-to-treat (ITT) population and PD-L1 positive subgroups] and OS (tested in the ITT population; if the finding was significant, it would be tested in the PD-L1 (+) subgroup). Stratification factors were: receipt or nonreceipt neoadjuvant or adjuvant taxane therapy, presence or absence of liver metastases at baseline, and PD-L1 expression at baseline (positive *vs* negative) according to immunohistochemical testing (Ventana SP142). The trial was initially designed to assign 350 patients for the evaluation of primary end point (PFS), but during the course of trial, enrollment was expanded to about 900 patients to accommodate the addition of OS as a second primary end point. 41% of the patients were PD-L1 (+) [11]. The possible rationale for using taxane-based chemotherapy is that it can enhance tumor antigen release and antitumor response to checkpoint inhibitors. Furthermore, nab-paclitaxel can promote dendritic cell activity and was used to avoid the interaction between atezolizumab and corticosteroids (under the rationale that the use of corticosteroids could decrease the immune response of anti-PD-L1 therapy). In addition, nab-paclitaxel has a decreased risk of hypersensitivity reactions and does not require corticosteroid treatment[12].

After a median follow-up of 12.9 mo in the ITT population, the addition of atezolizumab to nab-paclitaxel increased the median PFS (7.2 mo with atezolizumab + nab-paclitaxel *vs* 5.5 mo with placebo + nab-paclitaxel, hazard ratio [HR]: 0.80, 95% confidence interval [CI]: 0.69-0.92, P = 0.002), although this did not increase OS (21.3 mo with atezolizumab + nab-paclitaxel *vs* 17.6 mo with placebo + nab-paclitaxel, HR: 0.84, 95%CI: 0.69-1.02, P = 0.08). However, in the subgroup of PD-L1 (+) patients (defined as PD-L1 expression in tumor infiltrating immune cells ≥ 1% of the tumor area), the median PFS (7.5 mo *vs* 5.0 mo, HR: 0.62, 95%CI: 0.49-0.78, P < 0.001) and OS (25 mo *vs* 15.5 mo, HR: 0.62, 95%CI: 0.45-0.86) was improved with the combination of atezolizumab + nab-paclitaxel compared to placebo + nab-paclitaxel[11].

Regarding adverse events, the frequency of grade ≥ 3 adverse events (AEs) was 48.7% in the atezolizumab + nab-paclitaxel group and 42.2% in the placebo + nab-paclitaxel group, with neutropenia (8%), peripheral neuropathy (6%), fatigue (4%) and anemia (3%) being the most common events in both groups. Grade ≥ 3 immune-related events (irAEs) occurred in 7.5% and 4.5% of the atezolizumab + nab-paclitaxel and placebo + nab-paclitaxel groups, respectively. Authors conclude that atezolizumab + nab-paclitaxel prolonged PFS among patients with mTNBC in both ITT population and PD-L1 (+) subgroup [11].

An OS data update from a second interim analysis of a median follow-up of 18 mo showed an OS of 21.0 mo in the atezolizumab + nab-paclitaxel group *vs* 18.7 mo in the placebo + nab-paclitaxel group (P = 0.0777) on ITT. In the PD-L1 (+) subgroup, OS was 25.0 mo *vs* 18.0 mo (HR: 0.71). This update confirms the benefit in OS of the population with PD-L1 (+)[13]. Very recently, a final OS analysis from the IMpassion130 trial was published: final OS data from IMpassion130 agree with prior interim analysis. The OS benefit in the ITT population was not statistically significant (21.0 mo *vs* 18.7 mo, HR: 0.87, 95%CI: 0.75-1.02, P = 0.077). Data showed clinically meaningful OS benefit with the combination of atezolizumab + nab-paclitaxel in the PD-L1 IC-positive population (25.4 mo *vs* 17.9 mo, HR: 0.67, 95%CI: 0.53-0.86), 3-year OS rates in the PD-L1 group were 35.8% using atezolizumab + nab-paclitaxel *vs* 22.2% in the placebo group and no new safety events were reported with longer follow-up. The authors conclude that although OS benefit in the ITT population was not statistically significant, a clinical meaningful OS benefit was reported in PD-L1 IC-positive patients with atezolizumab + nab-paclitaxel. The statistical results of this trial (ITT population) were negative[14].

In conclusion, the combination of atezolizumab plus nab-paclitaxel prolongs PFS and OS in the mTNBC subgroup with PD-L1 (+) but not in the intention-to-treat (ITT) population, changing the treatment paradigm with patients in the metastatic setting. This combination has been initially included in international clinical practice guidelines (currently NCCN guidelines removed this option)[15] (IB, ESMO guidelines)[16] and the FDA (Food and Drug Administration) accelerated approval in March 2019 for its use in the treatment of patients with mTNBC or unresectable locally advanced disease with PD-L1 positive using a validated test[7]. This was the first approval of atezolizumab and of an immunotherapy regimen for the treatment of breast cancer[17]. It is important to note that the FDA has granted accelerated approvals to oncology medicines on the basis of evidence that suggests a benefit to patients, however many immunotherapies (atezolizumab, pembrolizumab, nivolumab, durvalumab) approval are under evaluation since the approval is based on a surrogate endpoint and it requires a confirmatory trial with a clear benefit. In addition, four indications were voluntarily withdrawn by manufacturers (nivolumab in metastatic small cell lung cancer, durvalumab in locally advanced or metastatic urothelial carcinoma, pembrolizumab for metastatic small cell lung cancer and atezolizumab for metastatic urothelial carcinoma)[18]. Although in April 2021 the FDA Oncologic Drugs Advisory Committee

(ODAC) voted 7 to 2 in favour of maintaining accelerated approval of atezolizumab in combination with nab-paclitaxel for the treatment of adults with unresectable locally advanced or mTNBC whose tumours express PD-L1. In August 2021, the manufacturer announced that it was voluntarily withdrawing atezolizumab indication for BC in United States. Due to recent changes in the treatment landscape (including IMpassion131 results) the FDA will no longer consider it appropriate to maintain the accelerated approval for atezolizumab in BC. The indication received accelerated approval based in benefit in PFS and OS of IMpassion130, but there was no difference in survival advantage in PD-L1 (+) nor ITT population of IMpassion131[19,20].

Ventana SP142: In the IMpassion130 study, not only was the approval of atezolizumab in combination with chemotherapy achieved, but the FDA also approved the antibody diagnostic measurement test "Ventana PD-L1 SP142 assay", to select TNBC patients to receive treatment with atezolizumab, and perhaps it could be considered a predictive biomarker[21]. Tumor samples were evaluated by immunohistochemistry to evaluate the expression of PD-L1 (Ventana SP142) in tumor infiltrating immune cells (PD-L1 IC), using a 2-level system: "a percentage of tumor area" < 1% (= PD-L1 negative) or > 1% (= PD-L1 positive). The study revealed that patients whose tumors were positive for PD-L1 (approximately 41%) and received atezolizumab + nab-paclitaxel had a better median PFS compared to placebo + nab-paclitaxel (7.2 mo *vs* 5.5 mo)[11]. In the PD-L1 (+) subgroup, the ORR was 59% with atezolizumab + nab-paclitaxel compared to 43% in the placebo + nab-paclitaxel group. Furthermore, 10% of the patients in the atezolizumab group achieved complete response (CR) compared to only 1% in the placebo group [17] (Table 2).

Atezolizumab + paclitaxel in TNBC (IMpassion131): IMpassion131, a phase III randomized trial, evaluated the combination of atezolizumab + paclitaxel compared with placebo + paclitaxel in patients with unresectable locally advanced disease or mTNBC who had not received prior therapy or ≥ 12 mo since neoadjuvant chemotherapy) (n = 651). Forty-five percent of patients were PD-L1 (+), 48% were treated with taxanes, 31% had mTNBC, and 27% had liver metastases. The primary endpoint of IMpassion131 was PFS, and there was no significant difference in PFS between the atezolizumab group *vs* placebo in PD-L1 (+) patients: 5.7 mo *vs* 6.0 mo, respectively (HR: 0.82, P = 0.20) or in the ITT population: median PFS was 5.6 *vs* 5.7 in the atezolizumab and placebo groups, respectively (HR: 0.86). Even in the OS analysis, no benefit was demonstrated with atezolizumab in the ITT population or in the PD-L1 (+) population. Regarding AEs, grades 3-4 were similar in both groups (43% *vs* 49%)[22].

In IMpassion130 trial, atezolizumab + nab-paclitaxel did not improve OS in ITT but resulted in a "clinically significant" improvement in OS in PD-L1 (+) patients. The results of the IMpassion130 trial demonstrated the benefit of atezolizumab in combination with nab-paclitaxel. However, the results were divergent in the IMpassion131. Potential reasons for the divergent results between the two studies are under investigation. Tumor heterogeneity could be a reason. Other reasons could be the use of concomitant corticosteroids (necessary for paclitaxel infusion) may have a negative effect on the immunotherapy activity (checkpoint inhibitors); likewise, the differences in the study populations may have a role, as well as the cremophor associated with paclitaxel.

In July 2021, primary results from IMpassion131 have been published. Neither PFS or OS were improved with the combination of atezolizumab + paclitaxel in PD-L1 (+) nor ITT population. The baseline characteristics of the populations in both trials were similar, including median PFS in control groups (5.6 mo with paclitaxel alone *vs* 5.5 mo with nab-paclitaxel alone). Ongoing research may be valuable to explain possible reasons for the IMpassion131 results; authors said the lack of information on BRCA status could be a limitation, as imbalances between treatment arms for this prognostic biomarker may not be detected. In addition, findings from IMpassion131 differ with KEYNOTE-355 results, which evaluated pembrolizumab and more chemotherapy backbones (nab-paclitaxel, paclitaxel, gemcitabine/carboplatin). Despite the main goal of KEYNOTE-355 was similar to that of IMpassion131 and there were important differences regarding eligibility, statistical design, PD-L1 testing and chemotherapy regimens[22].

Atezolizumab + adjuvant chemotherapy (Impassion 030): A pending question is to determine the effectiveness of anti-PD-1/PD-L1 in the adjuvant setting. Several studies are underway including IMpassion030, a phase II study evaluating atezolizumab + adjuvant chemotherapy *vs* placebo + chemotherapy[23].

Pembrolizumab

Pembrolizumab monotherapy in mTNBC (KEYNOTE-119): Pembrolizumab showed antitumor activity and a manageable toxicity profile in TNBC in the umbrella study KEYNOTE-012 (June 2017), a phase Ib study that evaluated the use of immunotherapy in advanced solid tumors. In the subgroup of patients with TNBC, an ORR of 18.5%, a stable disease rate (SD): 25.9%, partial response (PR): 14.8% and complete response (CR): 3.7% rates were obtained[24].

Then, the KEYNOTE-086 (March 2019) phase II study, which evaluated the use of pembrolizumab for up to 2 years as a second or subsequent line of treatment in patients with mTNBC (that previously received anthracyclines and taxanes). The primary endpoint was ORR in the subgroup of patients with PD-L1 (+). As results, an ORR of 4.7%, SD of 20.6%, PR of 4.1% and CR of 0.6% were obtained. In the

Table 2 Common commercially monoclonal programmed death ligand 1 antibodies for immunohistochemical analysis to assess the expression of programmed death ligand 1 (considering Food and Drug Administration approvals)

PD-L1 antibody	Immunotherapy	IHC assay	Cut-off	Line
22C3	Pembrolizumab	DAKO	TPS ≥ 1%	1L
			TC ≥ 1%	2L
28-8	Nivolumab	DAKO	TC ≥ 1%	2L
SP142	Atezolizumab	Ventana	TC ≥ 50% and/or IC ≥ 10%	1L
			TC ≥ 1% and/or IC ≥ 1%	2L
SP263	Durvalumab	Ventana	TC ≥ 1%	1L maintenance, in unresectable stage III after chemoradiation therapy
	Nivolumab		TC ≥ 1%	2L
	Pembrolizumab		TC ≥ 50%	1L
73-10	Avelumab	DAKO	TC ≥ 1%	2L (not approved)

Notes: (1) Atezolizumab in combination with nab-paclitaxel is approved as 1L of treatment for patients with unresectable locally advanced or metastatic triple-negative breast cancer (mTNBC) whose tumors express programmed death ligand 1 (PD-L1) immune cells (IC) (PD-L1 stained tumor-infiltrating IC of any intensity covering ≥ 1% of the tumor area), as determined by a Food and Drug Administration (FDA) approved test (Ventana SP142); and (2) Pembrolizumab with chemotherapy is approved as 1L of treatment for patients with locally recurrent unresectable or mTNBC whose tumors express PD-L1 CPS ≥ 10, as determined by an FDA approved test (PD-L1 IHC 22C3 PharmDx). CPS: Combined positive score; IC: Immune cell; IHC: Immunohistochemistry; TC: Tumor cell; TPS: Tumor proportion score; 1L: First-line; 2L: Second-line; PD-L1: Programmed death ligand 1.

latter, the response was independent of PD-L1 expression [4.8% in patients with PD-L1 (+) *vs* 4.7% PD-L1 (-)][25].

Subsequently, the KEYNOTE-119 (September 2019), phase III, open-label, randomized study was presented which used pembrolizumab monotherapy (n = 312) *vs* single agent chemotherapy (n = 310) in previously treated mTNBC patients (1-2 prior systemic treatments). The patients were stratified in PD-L1 (+) and (-). The primary endpoint was OS in patients with a combined positive score (CPS) ≥ 10, patients with CPS ≥ 1, and all patients. Secondary endpoints were PFS, ORR and safety. As results, pembrolizumab did not improve OS in patients with CPS ≥ 10 or CPS ≥ 1. In an exploratory analysis of patients with CPS ≥ 20, the median OS was 14.9 mo *vs* 12.5 with chemotherapy (HR: 0.58, 95%CI: 0.38-0.88), no improvement in PFS was observed. Grade 3-5 AEs were 14% *vs* 36% with chemotherapy. In conclusion, this monotherapy treatment did not improve significantly as a second or third line of treatment for mTNBC *vs* chemotherapy, but it was well tolerated and had a lower toxicity than chemotherapy[26].

Pembrolizumab + chemotherapy in mTNBC (KEYNOTE-355): Since pembrolizumab monotherapy showed antitumor activity in mTNBC patients, the KEYNOTE-355 (December 2020), phase III, randomized study evaluated the addition of pembrolizumab to chemotherapy in previously untreated patients with inoperable disease or mTNBC (n = 847), in two groups: pembrolizumab (200 mg IV every 21 d) plus nab-paclitaxel (100 mg/m^2 on days 1, 8 and 15 of a 28-d cycle), paclitaxel (90 mg/m^2 on days 1, 8 and 15 of a 28-d cycle), or gemcitabine (1000 mg/m^2) with carboplatin (AUC 2 on days 1 and 8 of a 21-d cycle) *vs* placebo plus chemotherapy. The co-primary endpoints were PFS and OS, evaluated in the PD-L1 subgroup with CPS ≥ 10, CPS ≥ 1, and in the ITT population[27]. As results, among patients with CPS ≥ 10, the median PFS was 9.7 mo in the pembrolizumab group *vs* 5.6 mo in the placebo group (statistically significant) (HR: 0.65, 0.49-0.86, P = 0.0012). Among patients with CPS ≥ 1, median PFS was 7.6 mo *vs* 5.6 mo (HR: 0.74, 0.61-0.90, P = 0.0014) (not significant) and in the ITT population, median PFS was 7.5 mo *vs* 5.6 mo (HR: 0.82, 0.67-0.97). The effect of pembrolizumab was increased in the enriched PD-L1 population (CPS ≥ 10). In the subgroup analysis, in the ITT population there was more benefit when pembrolizumab is used with paclitaxel, followed by nab-paclitaxel and gemcitabine/carboplatin, showing an asymmetry of chemotherapy regimens used with anti-PD-1 therapy. Similar results were observed in the population with CPS ≥ 1. Regarding AEs, grades 3-5 were 68% in the pembrolizumab group *vs* 67% in the placebo group, including death in < 1% in the pembrolizumab group *vs* 0% in the placebo group. In conclusion, pembrolizumab associated with chemotherapy showed a significant clinical improvement in PFS *vs* placebo in mTNBC patients with CPS of 10 or more[27].

The authors suggest a role in adding pembrolizumab to standard first-line chemotherapy in mTNBC. In fact, NCCN guidelines recommend pembrolizumab (associated to chemotherapy) as first-line treatment options in mTNBC (category 1, preferred as first-line therapy)[15].

It should be noted that, to date, ESMO guidelines do not recommend the use of immunotherapy in subsequent lines for mTNBC due to its low response rates (IB, ESMO)[16].

In the San Antonio Breast Cancer Symposium (SABCS) 2020, new findings from the KEYNOTE-355 trial were presented. Pembrolizumab plus chemotherapy improved PFS, ORR, durable CR and duration of response for patients with locally recurrent, unresectable or mTNBC with tumors expressing PD-L1 and a CPS ≥ 10. This additional endpoint results showed the PFS benefit for the addition of pembrolizumab to chemotherapy, regardless of which chemotherapy partner was chosen, particularly in PD-L1 enriched (CPS ≥ 10) patients[28].

In the ITT population, the median PFS in the pembrolizumab and placebo groups was 7.5 mo *vs* 5.4 mo when given with nab-paclitaxel, 8.0 mo *vs* 3.8 mo with paclitaxel, and 7.4 mo *vs* 7.4 mo with gemcitabine plus carboplatin. The hazard ratios (HRs) favored pembrolizumab over placebo, at a significant HR: 0.69 and HR: 0.57 for nab-paclitaxel and paclitaxel, respectively, and a nonsignificant HR: 0.93 for gemcitabine plus carboplatin. When stratified by PD-L1 expression, patients with a CPS ≥ 10 or CPS ≥ 1 had longer PFS with pembrolizumab. The trial was not powered to compare efficacy among treatment groups by different chemotherapy regimens[28].

In patients with CPS ≥ 10, secondary endpoints favored pembrolizumab plus chemotherapy compared with chemotherapy alone (ORR: 53.2% *vs* 39.8%, disease control rate: 65% *vs* 54.4%). The authors conclude these findings support a role of addition of pembrolizumab to standard chemotherapy for the first-line treatment of mTNBC[28].

In ESMO Congress 2021 (September 2021) final results from the KEYNOTE-355 confirmed pembrolizumab + chemotherapy met dual primary endpoints (PFS and OS) in patients with mTNBC whose tumors expressed PD-L1 (CPS ≥ 10). For all endpoints, the pembrolizumab effect increased with PD-L1 enrichment. No new safety signals were identified[29].

Recently, in SABCS 2021 (December 7-10th, 2021), final results of pembrolizumab plus chemotherapy in mTNBC were presented and demonstrated that the addition of pembrolizumab yielded significant survival over placebo. The authors suggested that a CPS ≥ 10 is considered a "reasonable" cutoff to determine expected treatment benefit[30].

PD-L1 IHC 22C3 pharmDx: The determination of PD-L1 status in the KEYNOTE-355 trial was assessed the PD-L1 IHC 22C3 pharmDx assay and characterized by the CPS, defined as the number of PD-L1 positive cell (tumour cells, lymphocytes and macrophages) divided by total number of tumour cells x 100. PD-L1 (+) tumours are classified as CPS ≥ 10 and CPS ≥ 1, and PD-L1 (-) tumours are classified as CPS < 1. The PFS and OS analysis in the KEYNOTE-355 trial was stratified using CPS ≥ 10, CPS ≥ 1 and the ITT population[27].

Based on KEYNOTE-355 results, in November 2020, the FDA granted accelerated approval to pembrolizumab (200 mg IV every 3 wk or 400 mg every 6 wk prior to chemotherapy) in combination with chemotherapy for the treatment of patients with locally recurrent unresectable or mTNBC whose tumors express PD-L1 (CPS ≥ 10) as determined by an FDA approved test. The FDA also approved the use of PD-L1 IHC 22C3 pharmDx (Dako North America Inc.) as a companion diagnostic test for selecting patients with TNBC who may be appropriate for treatment with pembrolizumab[31].

Pembrolizumab + neoadjuvant chemotherapy (KEYNOTE-522): Pembrolizumab associated to neoadjuvant chemotherapy demonstrated antitumor activity and safety in patients with early TNBC in the I-SPY 2 and the KEYNOTE-173 studies. The I-SPY 2 (September 2017), phase II randomized study designed to test new treatments by identifying therapies based on molecular characteristics included patients with HER2 negative, stage II - III breast cancer who were randomized to receive weekly neoadjuvant paclitaxel with or without pembrolizumab (200 mg IV every 3 wk x 4 cycles) followed by AC (every 3 wk x 4 cycles). In the TNBC subgroup (n = 118), it was demonstrated that the combination in the neoadjuvant setting increases pCR up to 3 times more (62.4% *vs* 22.3%, respectively) compared to the control[32].

Subsequently, the results of the KEYNOTE-522 (August 2020), phase III study, which included patients with non-metastatic TNBC, without previous treatment (n = 1174), were randomized 2:1 to receive pembrolizumab (200 mg every 3 wk) or placebo, both given with 4 cycles of paclitaxel + carboplatin, followed by 4 cycles of doxorubicin or epirubicin + cyclophosphamide (neoadjuvant phase). After surgery, patients received either pembrolizumab or placebo for 9 cycles until recurrence or unacceptable toxicity (adjuvant phase). The co-primary endpoints were pCR and event-free survival (EFS). As results, a pCR was achieved in 64.8% of the pembro group *vs* 51.2% with placebo (P < 0.001). The benefit in pCR with pembrolizumab was consistent across all subgroups, including those with PD-L1 (+). After a median of 15.5 mo, 7.4% of the pembro group and 11.8% of the placebo group had disease progression, local or distant recurrence, or death from any cause (HR: 0.63). The safety of pembrolizumab was consistent with previous studies. In conclusion, pCR was higher in patients receiving pembro + neoadjuvant chemotherapy compared with placebo[33]. A post-hoc analysis showed a better pCR difference in pembrolizumab group *vs* placebo group in clinical stages (CS) IIIA (66.7% *vs* 42.1%, Δ 24.6) and IIIB (48.6% *vs* 23.1%, Δ 25.6), also a better pCR difference by lymph node involvement: positive (64.8% *vs* 44.1%, Δ 20.6) *vs* negative (64.9% *vs* 58.5%, Δ 6.3).

An update of the KEYNOTE-522 trial (presented in ESMO virtual plenary, 15-16 July 2021) showed that at the median follow-up of 39 mo, pembrolizumab had a statistically and clinically significant EFS benefit (HR: 0.63, 95%CI: 0.48-0.82, P = 0.0003) compared with chemotherapy alone. At a 3-year follow-up, EFS was 84.5% in the pembrolizumab group compared with 76.8% in the placebo group. The most common event was distance recurrence (7.7% with pembrolizumab group *vs* 13.1% with placebo group). Moreover, pembrolizumab showed a favorable trend in overall survival (OS) (HR: 0.72, 95%CI: 0.51-1.02). Regarding the adverse events (AEs), the immune-mediated AEs (IMAEs) of any grade were found in 43.6% of pembrolizumab group *vs* 21.9% in the placebo group. The most common AEs reported with pembrolizumab were infusion reactions and hypothyroidism[34]. Based on results of the KEYNOTE-522, on July 2021, the FDA approved pembrolizumab for high-risk early-stage TNBC in combination with chemotherapy as neoadjuvant treatment. This is the first immunotherapy approved for early-stage TNBC[35].

DIFFERENCES BETWEEN IMPASSION130 AND KEYNOTE-355 TRIALS IN mTNBC

To clarify, the IMpassion130 and KEYNOTE-355 trials have similar designs and results are consistent. The overall survival results are expected to be similar and the benefit was in PD-L1 (+) patients, suggesting that the direction is identifying the presence of a biomarker (PD-L1 status).

One difference is the way the authors define PD-L1 (+) by immunohistochemistry at a central laboratory and the companion diagnostic methods: in IMpassion130 trial they look at PD-L1 expression of ICs (IC score: greater than 1% of the area of tumor, using SP142 assay), meanwhile, in the KEYNOTE-355, the authors used a different antibody (22C3) to look at PD-L1 expression (CPS: a combination of PD-L1 staining on ICs and the tumor cells, looking for immune cell staining and tumor cell staining greater than 10% of the area). SP142 was seen in 41% of PD-L1 (+) patients but the 22C3 is much higher (is close to 80%)[13,29]. Diagnostic companions can link or homogenize cut-off points of validated tests in order to obtain similar results.

Another difference is that the KEYNOTE-355 includes several standard chemotherapy regimens as taxanes (paclitaxel, nab-paclitaxel) or gemcitabine-carboplatin (IMpassion130 only used nab-paclitaxel as chemotherapy regimen) and patients with early recurrences, thereby offering more treatment options to a population with a high unmet medical need. It is important to note that the KEYNOTE-355 trial was not designed to compare chemotherapy regimens but the last update shows a trend of benefit using taxanes instead of gemcitabine-carboplatin in addition to pembrolizumab[29].

EMERGING BIOMARKERS OF RESPONSE TO IMMUNOTHERAPY IN TNBC

The results obtained in the PD-L1 (+) subgroup of the IMpassion130 trial confirm the benefit of immunotherapy in mTNBC. However, PD-L1 is not the ideal biomarker to select patients for anti-PD-1/anti-PD-L1 therapies as it has been shown in other cancers. Therefore, there is an urgent need for identification and implementation of emerging biomarkers that can predict response to immunotherapy.

TILs

High levels of TILs have been shown to have a prognostic value in patients with HER2 (+) breast cancer and TNBC, as a predicting factor of pathological complete response (pCR) to chemotherapy and its high expression seems to be linked to a better prognosis after adjuvant therapy as well as a reduction in the risk of recurrence[36].

TILs are frequently present in TNBC (around 20%) and they are associated with a good prognosis[37,38]. The characterization of the immune lymphocytic infiltrates, with the presence of a high number of T lymphocytes (CD8+ TILs), defines a better prognosis for neoadjuvant (higher pCR) and adjuvant chemotherapy (higher DFS and OS). The evidence indicates that in the neoadjuvant setting of TNBC, intratumoral TILs, as well as stromal ones, are predictive of pathological response to platinum-based chemotherapy[39]. However, currently, TILs score should not be used to make treatment decisions nor to escalate or de-escalate. TILs score can be used as a prognostic marker, providing a relative improvement of 15% to 20% in survival due to a 10% increase in TILs, and its use as a prognostic factor is supported by the 2019 St. Gallen Consensus[40,41].

Various studies on neoadjuvant and adjuvant therapies have measured TILs both at the intratumoral and stromal levels[42]. Some studies used immunohistochemistry while others evaluated molecular markers using immunohistochemistry and gene expression. At present, there is no specific cut-off point for TILs (+) established[43,44].

Stromal TIL score

A biomarker of interest is the stromal TIL score which is known to be prognostic and predictive in the

neoadjuvant setting. In the IMpassion130 analysis, the stromal TIL score or CD8+ cell count (T cells) did not predict the benefit of the use of atezolizumab. It also appears that a dearth of stroma in metastatic breast cancer samples could contribute to an inability to detect an association between stromal TILs and the benefit of atezolizumab[45]. Another study that compared the number of TILs in primary and metastatic tumors showed that TILs decrease in metastasis compared to primary breast tumors[46].

PD-L1

PD-L1, which can be expressed in tumor cells and/or in tumor infiltrating immune cells, contributes to the inhibition of the antitumor immune response in the tumor microenvironment[47].

TNBC can present a higher expression of PD-L1 (in a range of 21-56%) compared to the other subtypes, predominantly in inflammatory immune cells and occasionally in neoplastic cells[48].

PD-L1 expression is considered a useful biomarker of response to treatment pf anti-PD-1 or anti-PD-L1 therapies[49]. PD-L1 expression in immune cells (IC) has been estimated in a range from 40%-65% in TNBC patients[50,51]. In the IMpassion130 trial, the expression of PD-L1 IC ≥ 1% was used to define PD-L1 (+)[11].

It has recently been shown that the expression of PD-L1 IC along with TILs influence the prognosis of TNBC and can predict the response to immunotherapy with pembrolizumab and atezolizumab in breast cancer[52]. In the KEYNOTE-086 study, TNBC patients with PD-L1 (+) IC and high TILs had a better response to immunotherapy[53]. Furthermore, an exploratory analysis of the KEYNOTE-173 study investigating the combination of pembrolizumab and neoadjuvant chemotherapy in TNBC, shows that high levels of stromal TILs prior to treatment and the expression of PD-L1, reported in a combined score, were significantly associated with a higher pCR and overall response rates in TNBC patients who received chemotherapy and immunotherapy combined[54,55].

PD-L1 detection in tumor cells and immune cells (IC) varied by antibody clone and is easily evaluated using IHC. The most common commercially available monoclonal PD-L1 antibodies for immunohistochemical analysis to assess the expression of PD-L1 are the following: 22C3, 28-8, SP142, SP263 and 73-10. While many PD-L1 assays are available, only Ventana SP142 and PD-L1 IHC 22C3 pharmDx are licensed companion diagnostic tests for selecting patients with mTNBC who are candidates for treatment with atezolizumab and pembrolizumab, respectively[56].

Other emerging biomarkers in TNBC: PD-L1 has been mentioned as a biomarker to select patients to receive anti-PD1/PD-L1 therapies, being an imperfect marker as has been demonstrated in trials (in IMpassion031 and KEYNOTE-522 the benefit not confined to PD-L1 group). PD-L1 has some limitations: the difficult and subjective scoring (tissue types, cell types, antibodies), the expense for 22C3 validation for independent laboratories, the dependence on immune content of biopsy (number of immune cells), also it is not considered a great marker in most disease types. There is a great need for better predictive biomarkers for response to immunotherapy and many of them are under investigation, including: TILs, genetic signatures, TMB, microsatellite instability [microsatellite instability-high (MSI-H)/mismatch repair (MMR) deficiency], major histocompatibility complex (MHC) class I and II, *etc*.

TMB: The mutational burden of the tumor has been correlated with response to immunotherapy in various types of neoplasms; however, a high mutational burden is rare in breast cancer. In the study only 3.1% of breast cancers had high TMB (TMB-H) (≥ 10 mutations/Mb) when compared to 39.7% of melanomas and 24.3% of lung cancer[25]. TMB could be a potential biomarker in TNBC with TMB-H, but this could exclude patients that can benefit from immunotherapy[57,58].

TMB has an indication but clinically is not a great marker and is probably mostly driven by MSI.

MSI-H or deficient MMR: MSI-H or deficient MMR (dMMR) could be a predictive marker of response or benefit with anti-PD-1 therapy, taking into consideration that pembrolizumab is FDA approved for adult and pediatric patients with unresectable or metastatic, MSI-H or dMMR solid tumors that have progressed following prior treatment and who have no satisfactory alternative treatment options. However, MSI is infrequent in TNBC with an approximate frequency of 0.7%-2%[59].

Other potential biomarkers in TNBC: In view of the above, the research of new predictive biomarkers or risk factors (*e.g*., LDH levels, visceral liver disease) are underway to identify a group of patients that could benefit from atezolizumab as monotherapy or in combination, and thus optimize the treatment of mTNBC[60]. In the KEYNOTE-086 study, it was observed that patients with elevated levels of lactate dehydrogenase and visceral liver disease had little or no response to immunotherapy. Another study reports that patients with liver metastases derive limited benefit from immunotherapy independent of other established biomarkers of response: liver metastases create a systemic immune desert in preclinical models (apoptosis of CD8 T cells) and reduction of peripheral T cell numbers and diminished tumoral T cell diversity and function[61].

MHC-I and II are new potential biomarkers under analysis: most tumor cells (including BC) express MHC-I, whereas MHC-II is expressed by only a fraction of tumor/tumor cells (MHC-II is considered a professional antigen-presenting cell). A previous trial showed tumors which express high levels of MHC-I or II have high counts of CD4 and CD8 T lymphocytes ($P < 0.001$). Positive expression of MHC-II in tumor cells is associated with better disease-free survival (DFS) in patients who have lymph node

metastases (P = 0.009). Also, the expression of MHC-II in tumor cells was associated with an increased level of TILs[62]. A recent study reported MHC-II predicts early-stage HER2-negative breast cancer response to immunotherapy + neoadjuvant chemotherapy[63].

In general, the evolution of treatment with immunotherapy can be divided into "three waves":

The "first wave" includes the use of immunotherapy as monotherapy, which has shown antitumor activity and modest results in advanced disease.

The "second wave" used immunotherapy in combination with chemotherapy. Cytotoxic therapy can induce increased antigen release from tumor cells, change in tumor microenvironment, upregulation of PD-L1 and increased expression of cell surface markers (*e.g.*, MHC I). All of these effects can increase immunotherapy effectiveness. Despite evaluations of which would be the ideal (safest or most effective) chemotherapy for combination therapy with immunotherapy, several questions remain. Nab-paclitaxel was used in the IMpassion130 because it facilitates the reduction of corticosteroid use. However, other chemotherapy agents have also been evaluated to improve the immunogenicity of breast cancer, including anthracyclines, taxanes, platinum salts, among others[64].

The TONIC, phase II trial compared the effects of induction chemotherapy associated with immunotherapy (nivolumab). Objective response rate (ORR) was 20%, and the highest ORR rates were observed in the cisplatin (ORR: 23%) and doxorubicin (ORR: 35%) cohorts. Initial and post-induction biopsies analysis showed an upregulation of immune-related genes in PD-1/PD-L1 and T-cell cytotoxicity in the cisplatin and doxorubicin cohorts[65].

The lymphocyte depleting effect of combination therapy should also be considered. A comparison of chemotherapy (capecitabine or paclitaxel) associated with pembrolizumab showed a profound and significant depletion of T cells (including CD4+ and CD8+). This could explain the decrease in efficacy of anti-PD-1/PD-L1 in later lines of chemotherapy in TNBC[66].

The "third wave" includes immunotherapy in combination with targeted therapies (as PARP inhibitors). Currently, a phase II/III trial (KEYLYNK-009) of olaparib + pembrolizumab compared with chemotherapy (carboplatin/gemcitabine) + pembrolizumab after initial treatment with chemotherapy + pembrolizumab in TNBC (n = 932) is ongoing. The aim is evaluating if combination of olaparib and pembrolizumab is effective and safe. Co-primary endpoints are PFS and OS and results are ongoing[67].

In this setting, the use of antibody-drug conjugates (ADCs) can be included. Sacituzumab govitecan (a new ADC) is approved by the FDA for treatment of adult patients with mTNBC who received at least two prior therapies for metastatic disease based in results of ASCENT trial[68].

CONCLUSION

The treatment of TNBC has evolved in the last decade with the application of immunotherapy, which has become the new standard of treatment and is changing the management paradigm, mainly in advanced disease, where there were only limited treatment options such as systemic chemotherapy. Knowledge of the molecular profile of TNBC and immunogenicity has made it possible to identify characteristics that differentiate them from other subtypes. Likewise, immunotherapy was evaluated and approved for more TNBC scenarios (metastatic, neoadjuvant).

TNBC is considered a more immunogenic subtype compared to the other subtypes of breast cancer due to the higher expression of TILs and PD-L1. According to the analysis of IMpassion130, PD-L1 has been shown to be a discussible predictive biomarker of response in selected patients [subgroup with PD-L1 (+)]. Other potential biomarkers are under investigation (LDH levels, presence of visceral disease, TMB, MSI-H) to identify and select patients who may benefit from immunotherapy alone or in combination in the different scenarios of TNBC.

New advances have been made with immunotherapy in mTNBC. First, progression-free survival (PFS) and overall survival (OS) benefit have been demonstrated in selected populations (PD-L1 positive subgroups) with immunotherapy + chemotherapy (nab-paclitaxel) in metastatic stage (mTNBC), locally advanced or unresectable disease (IMpassion130 trial). Furthermore, the approval of anti-PD-1 also led to the approval of a companion diagnostic test (Ventana SP142) for selecting patients who are candidates for atezolizumab. However, the benefit of atezolizumab (PFS and OS) could not be demonstrated in combination with paclitaxel (study IMpassion 131). The reasons for the divergent results between IMpassion130 and IMpassion131 trials are currently under investigation. Second, the KEYNOTE-355 trial results are consistent with Impassion130 trial and pembrolizumab is considered as a first-line option of treatment in mTNBC. Moreover, there is another companion diagnostic test approved (PD-L1 IHC 22C3 PharmDx) as an aid to identify patients with TNBC who are candidates for pembrolizumab.

In the neoadjuvant setting of TNBC, pembrolizumab has achieved the 2 co-primary endpoints evaluated (KEYNOTE-522): a higher pCR when combined with chemotherapy and a statistically significant event-free survival (EFS) benefit compared with chemotherapy alone. In the metastatic setting, benefit has been shown with the use of pembrolizumab + chemotherapy (KEYNOTE-355 study) as the first-line of treatment in those patients with enriched expression of PD-L1 (CPS ≥ 10).

Finally, in adjuvant disease, ongoing studies (such as IMpassion030) are evaluating the benefit of immunotherapy. It should be noted that, for TNBC in early disease, the standard of treatment continues to be neoadjuvant chemotherapy, as this is considered a systemic disease.

The evolution of immunotherapy in TNBC began with immunotherapy as monotherapy ("first wave"), followed by combination of immunotherapy + chemotherapy ("second wave") that is considered the new standard of care as first line in selected mTNBC PD-L1 (+). Currently, there are ongoing trials evaluating the combination of immunotherapy (immune checkpoint inhibitors) plus targeted therapies (as PARP inhibitors) for several cancers including TNBC and the development of antibody-drug conjugates (as sacituzumab govitecan) which had demonstrated benefit in refractory mTNBC ("third wave").

FOOTNOTES

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